STUDY OF URINARY LEVEL OF KIDNEY INJURY MOLECULE-1 (KIM-1) IN ACUTE KIDNEY INJURY

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The work done by **DR. NEETHU VARGHESE** on **" STUDY OF URINARY LEVEL OF KIDNEY INJURY MOLECULE-1 (KIM-1) IN ACUTE KIDNEY INJURY"** is under my supervision and I assure that this candidate will abide by the rules of the Ethical Committee.

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CERTIFICATE

This is to certify that dissertation titled "**STUDY OF URINARY LEVEL OF KIDNEY INJURY MOLECULE-1 (KIM-1) IN ACUTE KIDNEY INJURY"** is a bonafide work done by **Dr.NEETHU VARGHESE** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2012 to 2015.

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DECLARATION

I, **Dr.NEETHU VARGHESE** hereby solemnly declare that the dissertation title "**STUDY OF URINARY LEVEL OF KIDNEY INJURY MOLECULE-1 (KIM-1) IN ACUTE KIDNEY INJURY"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department **Dr.N.Sasivathanam, M.D(Bio).,DGO.** This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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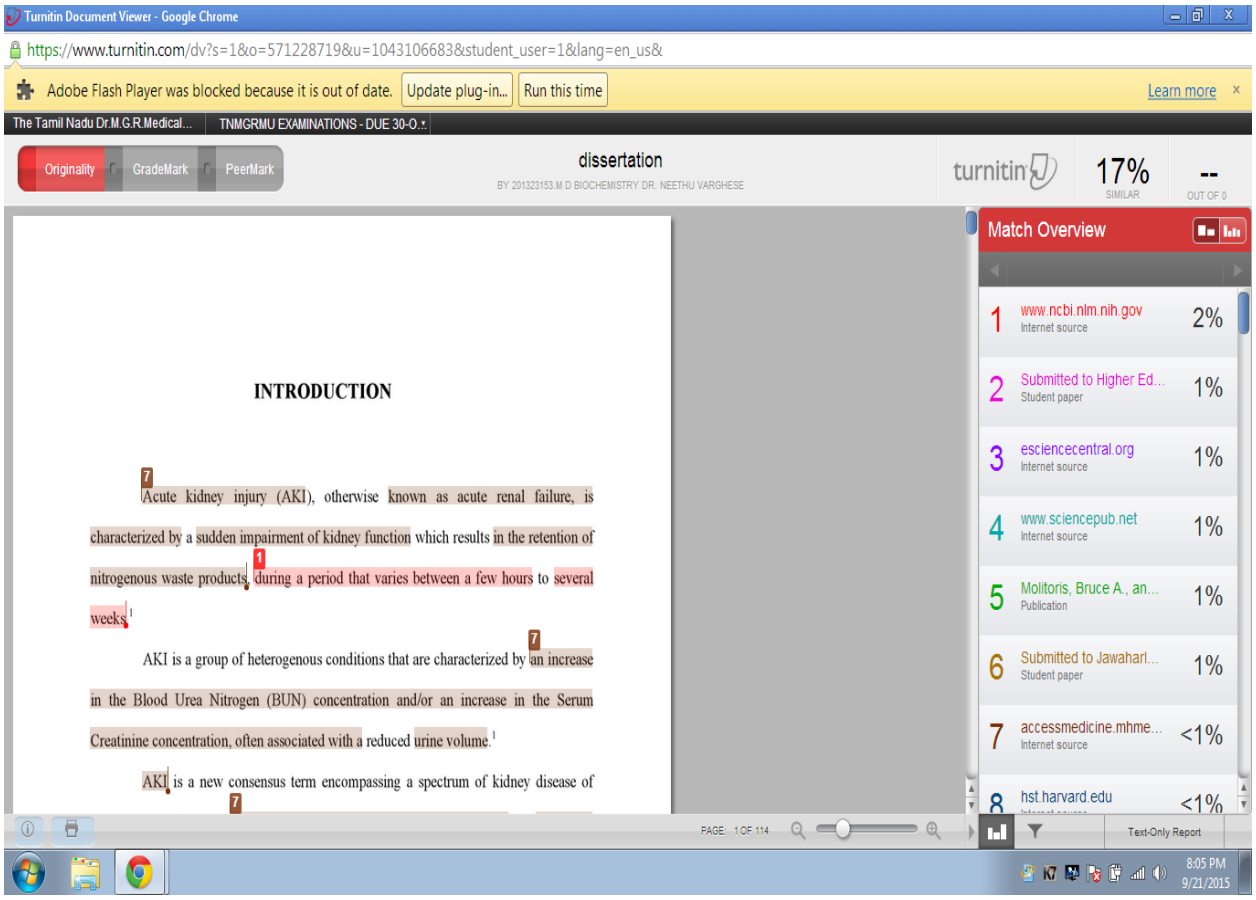

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CONTENTS

ABBREVIATIONS

AKI	: Acute Kidney Injury
BUN	: Blood Urea Nitrogen
ARF	: Acute Renal Failure
CKD	: Chronic Kidney Disease
KIM-1	: Kidney Injury Molecule -1
TIMS	: T-cell Immunoglobulin Mucin proteins
ATN	: Acute Tubular Necrosis
AKIN	: Acute Kidney Injury Network
ADQI	: Acute Dialysis Quality Initiative
GFR	: Glomerular Filtration Rate
RRT	: Renal Replacement Therapy
KDIGO	: Kidney Disease Improving Global Outcome
NSAIDs	: Non-steroidal Anti- Inflammatory Drugs
MCP-1	: Monocyte Chemoattractant Protein-1
IL-8	: Interleukin-8
ICAM-1	: Intercellular Adhesion Molecule-1
ATP	: Adenosine Triphosphate

ET-1	: Endothelin-1
PCT	: Proximal Convoluted Tubule
ADF	: Actin Depolymerisation Factor
TNF-α	: Tumor Necrosis Factor- α
ROS	: Reactive Oxygen Species
NO	: Nitric Oxide
LPS	: Lipopolysaccharide
DIC	: Disseminated Intravascular Coagulation
LDL	: Low Density Lipoprotein
ELISA	: Enzyme Linked Immuno Sorbent Assay

INTRODUCTION

Acute kidney injury (AKI), otherwise known as acute renal failure, is characterized by a sudden impairment of kidney function which results in the retention of nitrogenous waste products, during a period that varies between a few hours to several weeks.[1]

AKI is a group of heterogenous conditions that are characterized by an increase in the Blood Urea Nitrogen (BUN) concentration and/or an increase in the Serum Creatinine concentration, often associated with a reduced urine volume.[1]

AKI is a new consensus term encompassing a spectrum of kidney disease of acute onset, which may range in severity from asymptomatic changes in the laboratory parameters of glomerular filtration rate, to rapidly fatal changes in the regulation of effective circulating volume and composition of plasma electrolyte and acid-base balance.[1] Hence the term AKI has replaced the term acute renal failure (ARF) since AKI denotes the entire spectrum of clinical features ranging from mild increase in serum creatinine levels to overt renal failure.[2]

There is an enormously growing burden of AKI which is associated with an increased risk of mortality among those who are hospitalized, especially in those admitted to the Intensive Care Unit.[1] The incidence of hospital-acquired AKI has been increasing from the past few years which has been validated within a single patient population from 1996 through 2003.

Despite the advances in dialysis and intensive care, the mortality rates continue to be high from 30 to 65%. AKI was not only associated with an increased mortality, but also an increase in the hospital stay period and the overall cost.[2] The long term consequences of AKI include the risk of ending up in CKD stages 4 and 5.

The assessment of renal function traditionally involves the measurement of serum blood urea nitrogen and serum creatinine which are insensitive and nonspecific, to adequately detect renal injury prior to significant loss of renal function.[3] And also it is important to note that serum blood urea nitrogen and creatinine are not true 'injury markers', but are primarily markers of 'functional changes in filtration capacity'.

Of late, there is an urgent need of biomarkers for AKI for the timely diagnosis, and to predict the severity and outcome of AKI.

Kidney Injury Molecule-1 (KIM-1) is a recently discovered transmembrane protein found in the renal tubules, that are not detectable in the normal kidneys, but are markedly induced and expressed following renal injury including AKI.[4]

KIM-1 belongs to a large family of KIMs, otherwise known as T-cell Immunoglobulin Mucin proteins (TIMS), which functions as a signal or receptor for adhesion or signaling.[5]

Following rapid cleavage of the protein from the apical membrane of the tubular epithelial cell into the tubular lumen, it becomes detectable in the urine much earlier than the increases in serum blood urea nitrogen and creatinine.[4]

The present study was done to assess the levels of urinary Kidney Injury Molecule – 1 in patients with Acute Kidney Injury and to assess its utility as an early biomarker of AKI.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Acute kidney injury (AKI) is a clinical syndrome, which is characterized by a rapid decline in the glomerular filtration rate over hours to days .The term acute kidney injury or impairment encompasses the entire spectrum that ranges from minimal elevations in the serum creatinine level, to complete anuric kidney failure.[6]

AKI is a common complication of critical illness, associated with an increased risk of future chronic kidney disease, end stage renal disease and long term mortality, and it has an independent effect on the risk of death.[7]

HISTORICAL TRENDS

The first description of ARF, which was then termed as ischuria renalis, was done by William Heberden in the year 1802. With the commencement of the twentieth century, ARF, then termed as Acute Bright's disease, was described in the Textbook of Medicine by William Osler (1909), as a consequence of toxic agents, burns, pregnancy, operations or trauma in the kidneys.[8]

Following the First World War, AKI was named ‘war nephritis’. The syndrome was forgotten until the World War II , when a paper on crush injury was published by Bywaters and Beall. AKI due to Acute Tubular Necrosis (ATN) was recognized in the 1940s in the United Kingdom, where crush injury victims during the London Blitz developed patchy necrosis of renal tubules leading to a sudden decrease in the renal function.

However, the credit for the introduction of the term ‘acute renal failure’ was awarded to Homer W.Smith in a chapter on ‘Acute renal failure related to traumatic injuries’ in his textbook, The kidney – structure and function in health and disease (1951).[8]

EPIDEMIOLOGY

Of late, the 20th century has witnessed a high prevalence of AKI and mortality associated with it.

The incidence of AKI is dependent on the clinical setting as well as the definition that has been used in the analysis.[2] As the medical care becomes more and more complex, the likelihood of acquiring nosocomial AKI would continue to shoot up.

The incidence of AKI in the population is increasing at the rate of more than 7% per year[9]. Each year about 2 million people die of AKI[10]. It was estimated that over 1

of 5 hospitalized patients ended up developing AKI and an associated fourfold increase in the mortality was observed.[6]

A multinational epidemiological study was conducted at 54 centres in 23 nations in North and South America, Asia, Europe, and Australia, where 29,000 patients who were hospitalized in the intensive care unit were assessed. The incidence of AKI over a period of 16 months was found to be 5.7% (1.4% to 25.9%) out of which about 58.9% of patients were hospitalized for medical and 41.1% for surgical problems.[9]

AKI is very common following cardiac surgery, which represents about 25.2% of all cases. Overall, the hospital mortality was found to be 60.3%.[11] It was estimated that around 36% of cases with AKI required renal replacement therapy.

In children, Hemolytic Uremic Syndrome was the most common cause of AKI, followed by gastroenteritis and glomerulonephritis causing acute tubular necrosis.

In elderly patients aged above 60 years pre-renal causes of AKI among hospitalized patients account for 58% of all AKI cases. Post renal causes in the community are more common. The overall mortality is 54%. Mortality is higher (59%) in patients developing AKI during hospitalization when compared to AKI in the community(41%).[11]

The epidemiology of AKI in developing countries differs when compared to developed countries in many ways. The incidence of AKI in developing countries is higher than in the developed areas.[12] In developing countries, the presentation of AKI is bimodal. In large, urban areas, AKI is a hospital acquired disease, which occurs more commonly among older, critically ill patients with multiorgan failure and co-morbidities.

The main cause of AKI includes ischemia due to sepsis and nephrotoxic drugs. On the other hand, in rural areas AKI is usually a community acquired disease, mainly affecting younger individuals. The causes include diarrheal diseases, infectious diseases, scorpion stings and septic abortions.[10]

In developed countries, AKI is a disease of elderly with multiple co-morbidities. Causes include overdosage of nephrotoxic drugs, hospital acquired infections, sepsis and complex diagnostic procedures involving intravenous contrast.

CURRENT SCENARIO OF AKI IN INDIA

India being a developing country, gastroenteritis and infections are the most common causes of AKI.[13]

Lack of personal hygiene, poor socioeconomic status, lack of supply of clean water, overcrowding and lack of adequate medical facilities contribute to the high prevalence of AKI in our country.[13]

In a recent publication from Institute of Medical Sciences, BHU in India, it was found that over a period of 26 years from 1983 to 2008, the incidence of ARF increased from 1.95 per 1000 admission in the period of 1983 to 1985, to 4.19 per 1000 admission during 1996 to 2008.[14] In India, it was found that the most common cause of AKI in the community is medical (77.5%). Other major causes were obstetric (14.2%) and surgical causes(8.3%).[15]

Acute diarrheal diseases were the most common among medical causes. Nephrolithiasis was the most common surgical cause. Obstetric causes included puerperal sepsis, intrauterine death, intra-partum and post-partum hemorrhage.[15]

Sepsis was found to be the most common contributor of in hospital mortality, which was most commonly complicated by urogenital infections.[16]

The most common co-morbidities associated with AKI were found to be Type 2 Diabetes, followed by Hypertension and Coronary Artery Disease.[15]

DEFINITION OF ACUTE KIDNEY INJURY

Acute kidney injury is defined as a heterogenous syndrome of abrupt (within hours to days) decline in the kidney function that leads to retention of nitrogenous waste products such as urea and creatinine, dysregulation of fluid, electrolyte and acid-base homeostasis, often associated with a reduced urine output.[2,14]

DIAGNOSIS OF ACUTE KIDNEY INJURY

Acute Kidney Injury Network (AKIN) was established by a network of international experts who represented most of the nephrology societies in the world. This network included representatives from Acute Dialysis Quality Initiative (ADQI) group which developed an acceptable staging system called RIFLE for AKI, based on severity of kidney injury.[17]

The acronym RIFLE indicates

R - risk of renal dysfunction

I - injury to the kidney

F - failure of kidney function

L - loss of kidney function

E - end stage renal disease[7]

RIFLE is a multilevel classification system in which the grades of severity in the first three levels are based on concentration of serum creatinine, decrease in the urine output from the baseline and degree of reduction in GFR.[11,14] The next two levels depict the clinical outcomes based on the need for renal replacement therapy (RRT), reflecting the severity of prognosis.[11] This system can detect patients with mildly affected renal function, which means a high sensitivity but limited specificity, and in those in whom there is marked renal dysfunction (high specificity but limited sensitivity for detection).[7]

Risk-Injury-Failure-Loss-ESRD (RIFLE) and Acute Kidney Injury Network (AKIN) criteria in Acute Kidney Injury

RIFLE	**AKIN**	**CRITERIA**	
Risk	Stage 1	Increased creatinine ×1.5 or GFR decrease >25% (for AKIN the definition also met if creatinine increase is ≥0.3mg/dl.	Urine output <0.5ml/kg/hr × 6hr
Injury	Stage 2	Increased creatinine ×2 or GFR decrease >50%	Urine flow rate <0.5ml/kg/hr ×12hr
Failure	Stage 3	Increased creatinine ×3 or	Urine output

		GFR decrease >75% or creatinine >4mg/dl (for AKIN definition also met if renal replacement therapy initiated)	<0.3mg/kg//hr×24 hr or anuria × 12hr.
Loss	Persistent AKI with complete loss of kidney function and requiring RRT for > 4 weeks		
ESRD	End-stage renal disease requiring dialysis for >3months		

Recently it was found that even small increases in creatinine of ≥0.3mg/dl were associated with adverse outcomes and increased mortality. In 2007, the AKIN therefore developed a classification of AKI, which was a modified form of the RIFLE classification[18]. The Risk, Injury, Failure levels became stages 1, 2 and 3. Stage 1 also included a rise in ≥0.3mg/dl of creatinine within 48hours. The criteria based on GFR was removed. The Loss and ESRD stages were also removed because they were considered as outcomes and not stages.[19]

The recent KDIGO (Kidney Disease Improving Global Outcome) guideline for AKI stipulated yet another definition for AKI as any of the following:

- Rise in serum creatinine by ≥0.3mg/dl within 48 hours
- Rise in serum creatinine × 1.5 from the baseline which has occurred within the past 7 days
- A urine output of <0.5ml/kg/hr for 6 hours.[1]

These new definitions had many disadvantages because these were based entirely on the increases in serum creatinine or reduction in urine output, rendering

the timely diagnosis of AKI difficult.[20] There was incoherence between serum creatinine concentration and baseline GFR, different levels of serum creatinine between patients with the same extent of renal injury and delayed increases in serum creatinine relative to the onset of kidney injury.[11]

PATHOPHYSIOLOGIC CLASSIFICATION OF AKI

AKI can be classified pathophysiologically, depending on whether it results from,

- Diseases causing renal hypoperfusion, yet with a preserved integrity of renal parenchymal tissue, the entity being termed as **pre-renal state.**
- Diseases causing direct renal parenchymal damage - **intrarenal or intrinsic AKI.**
- Diseases causing obstruction of urinary tract - **post-renal state.**[21]

ETIOLOGY OF ACUTE KIDNEY INJURY

I. PRE-RENAL CAUSES (40-70% of cases)

a) Hypovolemia : haemorrhage, vomiting, diarrhea, burns.

b) Renal hypoperfusion : NSAIDS, ACE inhibitors, abdominal aortic aneurysm, renal artery stenosis, hepatorenal syndrome.

c) Hypotension : cardiogenic shock, sepsis, anaphylaxis.

d) Oedematous states : cardiac failure, hepatic cirrhosis, nephrotic syndrome.

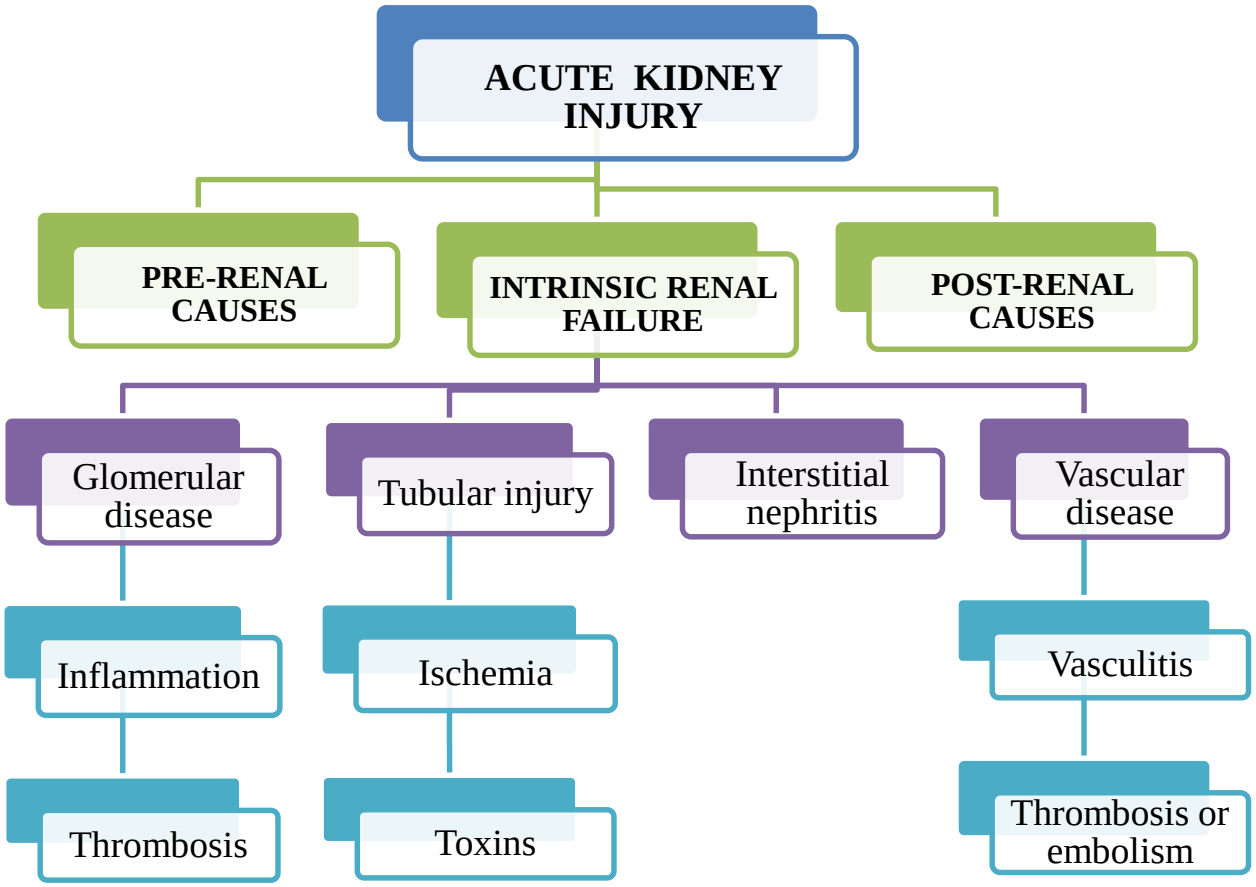
II. INTRINSIC RENAL CAUSES (10-50% of the cases)

a) Glomerular diseases : post-infectious glomerulonephritis, Henoch-Schonlein purpura, systemic lupus erythematosis, anti-glomerular basement membrane disease, disseminated intravascular coagulation.

b) Interstitial nephritis : NSAIDS, lymphoma, sarcoidosis, tuberculosis, pyelonephritis.

c) Tubular injury : ischemia, toxins – aminoglycosides, radiocontrast media, myoglobin, hypercalcemia, urate & oxalate crystals.

d) Vascular : vasculitis, cryoglobulinemia, polyarteritis nodosa, thrombotic microangiopathy, renal artery or vein thrombosis.



III. POST-RENAL CAUSES (10% of cases)

a) Intrinsic : intraluminal stone, blood clot, papillary necrosis, urethral stricture, prostate enlargement, bladder tumor, radiation fibrosis.

b) Extrinsic : pelvic malignancy, retroperitoneal fibrosis.[22]

RISK FACTORS INVOLVED IN THE GENESIS OF AKI

The most common risk factors include :

- advanced age,
- presence of chronic diseases such as diabetes, hypertension, heart disease and liver cirrhosis,[22]
- presence of infections and sepsis,
- pre-existing chronic kidney disease,[18]
- failing organs such as cardiovascular and respiratory failure,[23]
- surgery, mainly complex heart and vascular surgeries,
- mechanical ventilation[24] &
- intake of nephrotoxic drugs such as NSAIDs and aminoglycosides[25],

PATHOGENESIS OF AKI

Kidney is particularly prone to injury because of its characteristic blood supply and its capability of concentrating toxins.[26]

Pre-renal AKI

- **Autoregulation of renal blood flow :** When blood pressure falls, an intrinsic myogenic mechanism mediates vasodilation of afferent arterioles. To maintain a constant hydrostatic pressure at the glomerular capillaries, angiotensin II mediates vasoconstriction at the efferent arterioles.[27] Autoregulation occurs upto a mean arterial blood pressure of 80mmHg.

- However, when there is a marked reduction in perfusion to the kidney occurs, autoregulation is overwhelmed and results in renal ischemia ending up in acute tubular necrosis.[25,28]

Following renal ischemia, the molecular responses include the following :

a) Increased gene expression of :

Genes involved in cell fate determination (regeneration / apoptosis)

- Transcription factors : c-jun, c-fos
- Cyclin dependant kinase inhibitor

Genes involved in inflammation :

- Chemokines : MCP-1, IL-8
- Adhesion molecules : ICAM-1, integrins.

b) Decreased gene expression (loss of mature phenotype) of :

- Preproepidermal growth factor
- Tamm-Horsfall protein
- Aquaporin-2 [29]

- **Ischemic AKI :** Severe reduction in renal perfusion results in inability of tubular cells to maintain intracellular ATP. This leads to cytoskeletal disorganization, tubular epithelial tight junction disruption, endothelial cell injury, upregulation of adhesion molecules, shedding of components of glycocalyx, activation of leukocytes and coagulation pathway and inflammation. In severe cases, apoptosis or necrosis of the cells occur.[30]

Acute Tubular Necrosis

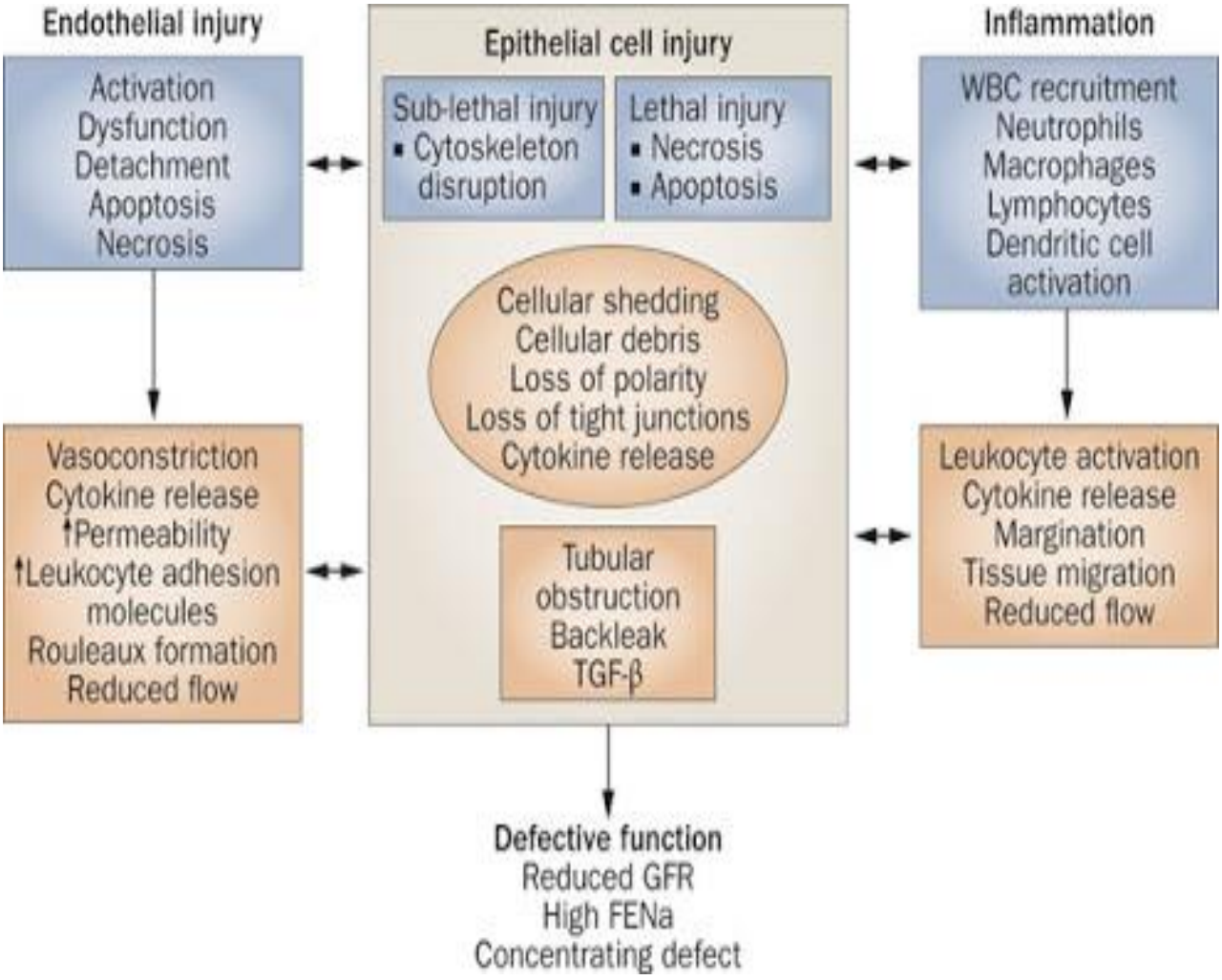
Morphological changes in the proximal tubular epithelium of kidney in response to ischemic or toxic insult include:

1. Loss of polarity and integrity of cytoskeleton
2. The damaged cells undergo necrosis and apoptosis
3. Surviving epithelial cells dedifferentiate
4. The poorly differentiated regenerating epithelial cells migrate and spread over the denuded basement membrane
5. Surviving proximal tubular epithelial cells proliferate
6. Ultimately, the regenerating epithelial cells redifferentiate to re-establish a fully functional normal proximal tubule epithelium.[31,32]

Tubular cell injury :

The most severe injury involves the outer medulla of kidney, specifically S3 segment of the proximal tubule and medullary thick ascending limb of distal nephron. Earliest damage occurs to the brush border of proximal tubular cells. The resulting loss in microvillar surface leads to an ineffective phagocytosis, transporter and channel density, hence a reduced transcellular absorption.[25] Loss of tubular epithelial cells forms gaps in the tubular architecture. Sloughed off tubular cells and denuded basement membrane form intratubular casts which can obstruct the tubular lumen.[21]

Figure 1 : Pathogenesis of AKI



The mediators of tubular cell injury include reactive oxygen species, intracellular calcium influx, phospholipase A_2, nitric oxide, complement and cell mediated cytotoxicity.[28]

Hemodynamic vascular alterations :

Renal injury can result in profound reduction in the GFR. This is due to afferent arteriolar vasoconstriction which causes a drop in the filtration pressure. This could be a result of endothelial cell injury, resulting in an imbalance in the vasoactive substances, with a predominant vasoconstrictive effect.[21] The vasoconstrictor substances which have been implicated in this include angiotensin II, adenosine, endothelin-1, thromboxane A_2, leukotrienes C_4 and D_4, prostaglandin H_2 and sympathetic nerve stimulation.[28]

Furthermore, ischemic and toxic injury increases the release of endothelin (ET-1) from endothelial cells, which is the most potent vasoconstrictor known. In AKI, the decreased neutral endopeptidase results in decreased degradation of ET-1, hence explaining its sustained increase.[27] The major effects of ET-1 include:

- Renal vasoconstriction and mesangial cell contraction
- Mitogenesis and proliferation of mesangial cells
- Recruitment and activation of leukocytes.[25]

In AKI, reduced sodium reabsorption by the proximal tubules increases the concentration of sodium chloride which is delivered to the macula densa. This activates the renin-angiotensin system resulting in a decline in GFR. This sequence of events is called tubuloglomerular feedback.[27]

Tubular obstruction :

Tamm-Horsfall protein can polymerise to form casts by further trapping the tubular cell debris and denuded basement membrane, causing tubular obstruction.[21]

Integrins are heterodimeric ($\alpha_3\beta_1$) glycoproteins which recognize the RGD sequence (arginine-glycine-aspartate) in matrix proteins, which mediates cell-to-cell adhesion. Oxidative stress causes depletion of integrins on the cellular basal surface, leading to loss of anchorage to the basement membrane and desquamation of cells. Movement of integrins from basolateral location to the apical membrane results in detatchment of tubular cells and further promiscuous adhesion of sloghed off cells to the cells remaining in situ, thus initiating tubular obstruction.[27]

Tubular backleak :

In the setting of loss of epithelial cells, loss of adhesion molecules (E-cadherins) and loss of tight junction proteins (ZO-1, occludin) , which maintain separation of tubular filtrate from surrounding interstitium, the glomerular filtrate which enters tubular space will leak back into the renal interstitium and gets reabsorbed into systemic circulation resulting in a decline in the effective GFR.[21]

Role of nitric oxide :

In AKI, cytokines induce the expression of iNOS leading to an increase in the production of nitric oxide. On the other hand, secondary to endothelial dysfunction eNOS

is inhibited. This imbalance between eNOS and iNOS, with a relative decrease in eNOS, increases susceptibility to microvascular thrombosis due to loss of thrombogenic property of endothelium, enhances neutrophil adhesion to the endothelium and vasoconstriction.

Increase in iNOS induces tubular epithelial cell injury, enhances motility of neutrophils and vasomotor response is attenuated.[25]

Following ischemia-reperfusion injury, generation of NO and superoxide radicals result in the formation of peroxynitrite ($OONO^-$) which is cytotoxic causing lipid peroxidation and DNA damage.[27]

Endothelial cell injury :

In response to acute renal ischemic and oxidant injury, endothelail cells undergo swelling, overexpression of adhesion molecules (ICAM-1) with an increased leukocyte - endothelial cell interaction and blunting of vasorelaxation due to decreased eNOS and vasodilatory prostaglandins.[28] The normal architecture of endothelial barrier is lost creating gaps between confluent endothelial cells due to loss of tight junctions. The normal actin cytoskeleton of endothelial cells is lost due to ATP depletion which reversibly disrupts F-actin structures.[25]

Role of inflammation :

Neutrophils and other inflammatory cells play an important role in the pathogenesis of ischemic AKI. The series of events involving adherence of leukocytes to the vascular endothelium involves adhesion molecules including selectins, mucins, endothelins and immunoglobulin superfamily.[25]

The earliest feature of inflammation is margination of neutrophils to the vascular endothelium. Activation of leukocytes to release cytokines involves reception of signals through circulating chemokines or through direct interaction with endothelium. Once activated, the leukocyte integrins undergo conformational change and bind to endothelial ligand for firm adhesion. This firm adhesion is imparted through interactions between endothelial cell integrins and ICAM-1.[21,25]

Role of inflammatory cytokines :

In response to acute ischemic or toxic injury, renal tubular epithelial cells express many proinflammatory cytokines which include TNF-α, interferon-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins 1, 2 and 18 ; and chemokines such as macrophage inflammatory protein-1 (MIP-1), monocyte chemotactic protein-1 (MCP-1), and RANTES, which promote infiltration of leukocytes.[25,28]

Disruption of actin cytoskeleton :

The actin cytoskeleton in PCT forms a terminal web layer below the apical membrane. A core of F-actin filaments extend from the web layer to the tip of microvilli, hence maintaining the integrity of brush border.

Ischemia results in ATP depletion in the cells, which in turn disrupts and redistributes the F-actin core to form membrane-bound vesicles or blebs. This disruption is due to depolymerisation mediated by actin binding protein called Actin Depolymerisation Factor (ADF) or cofilin, which is normally inactive in its phosphorylated form. Ischemia which causes ATP depletion dephosphorylates and

activates ADF. ADF then relocalises to the apical membrane and causes depolymerisation, capping, severing and F-actin nucleation.[33]

Actin binding proteins such as spectrin and ankyrin are degraded by the activation of cyateine protease calpain. This results in abnormal translocation of sodium-potassium-ATPase and other proteins subsequently resulting in loss of cellular polarity. This impairs the normal reabsorption of solutes with increased delivery of sodium chloride to the distal nephron and activation of tubule-glomerular feedback.[28]

Cell necrosis and apoptosis :

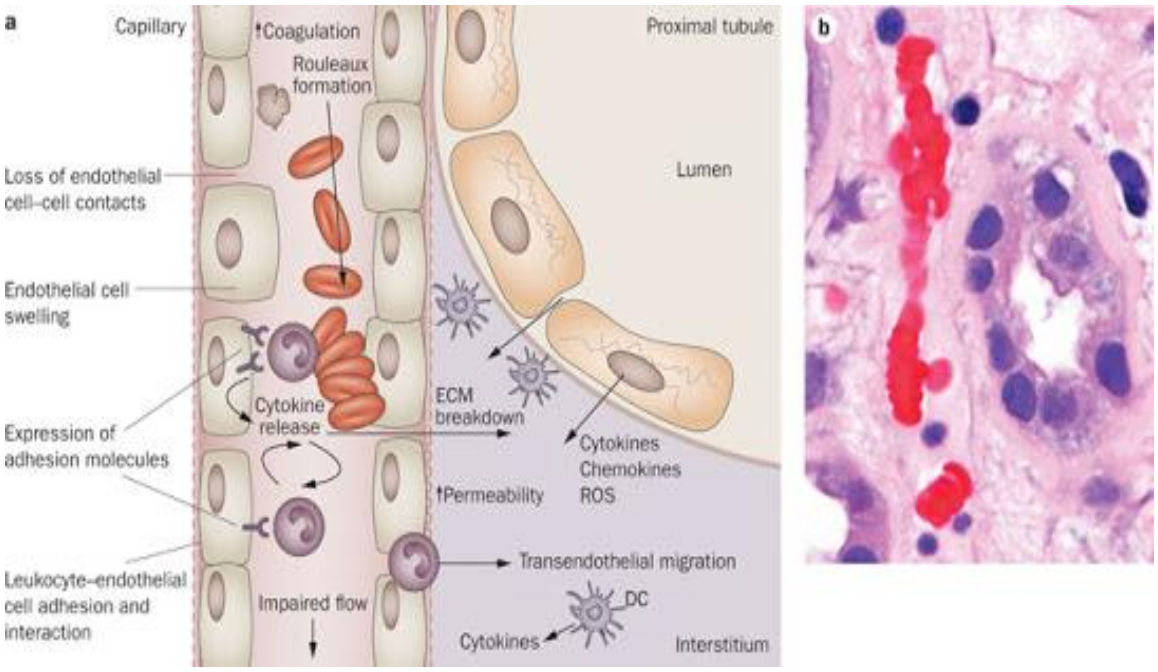
The severity and duration of renal tubular cell injury determines the mechanism of cell death. Cells suffering lethal injury undergo cell death by apoptosis or necrosis. Particularly necrosis occurs when there is severe depletion of energy stores of the cells with subsequent metabolic shutdown.

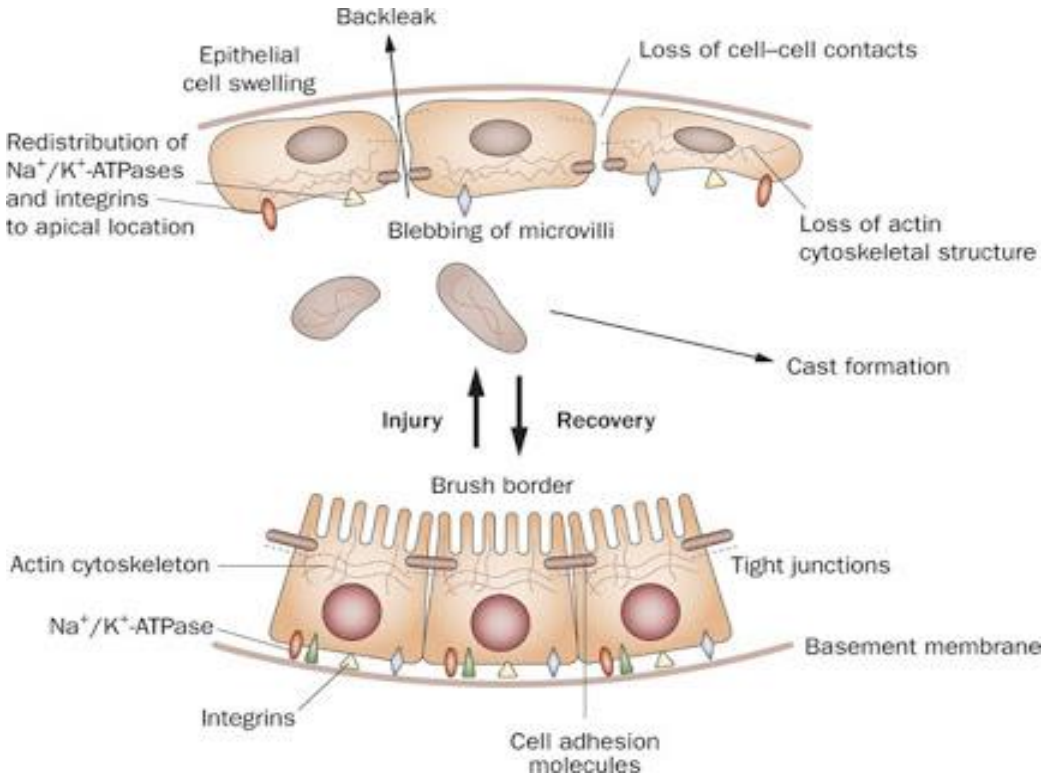
The biochemical mechanisms resulting in cell necrosis include:

- Severe intracellular ATP depletion
- Generation of reactive oxygen species
- Avtivation of enzymes such as phospholipases, proteases and endonucleases.

During apoptosis, mitochondria undergoes certain irreversible changes such as collapse of electron transport chain and release of apoptosis-inducing factor and cytochrome C which are indispensable for the activation of DNases and downstream caspases respectively.

Figure 2 : Role of inflammation and disruption of cytoskeleton





Post-renal AKI

Ureteric obstruction causes ARF when there is either a bilateral blockage at any level of ureters, or unilateral blockage in a solitary functioning kidney. Obstruction may be intraluminal or extraluminal. The most common cause is structural or functional obstruction of neck of the bladder.[25]

In the early stages, the glomerular filtration continues to be normal. This leads to an increase in intraluminal pressure of the renal tubules, upstream of the site of obstruction. The proximal ureter, pelvis and calyses gradually start distending, and eventually GFR declines. Furthermore, vasoconstriction of arterioles supervenes, further declining the GFR.[21]

CLINICAL COURSE OF AKI

I. **Initiation phase**

It is the phase wherein the patient is exposed to ischemia or toxins, with an evolving renal parenchymal injury, but not established, hence potentially preventable.[21] This period lasts for hours to days, and is characterized by :

- Loss of brush border, destruction of cytoskeleton
- Loss of cellular polarity, detatchment of tubular cells
- Formation of casts & reduction of glomerular filtration[11]

II. **Maintenance phase :**

This phase is characterized by an established parenchymal injury and GFR stabilizes at a value of 5-10ml/min. It typically lasts for about one to two weeks.[21] This phase is characterized by :

- Microvascular changes, imbalance between vasodilatory and vasoconstrictive substances
- Coagulation cascade activation and inflammation[11]

III. Recovery phase

This is the period when renal function starts recovering by regeneration of renal tissue. It is typically heralded by a gradual rise in urine output, fall in serum creatinine concentration and osmotic dieresis-induced by filtered urea.[21] Regenerating cells colonizing the injured areas can be derived from three sources:

a) Dedifferentiated surviving epithelial cells

b) Resident kidney stem cells

c) Bone marrow derived stem cells which migrate to the kidney and transdifferentiate to form mature tubular epithelial cells.[29]

ACUTE KIDNEY INJURY IN SPECIAL SITUATIONS

AKI FOLLOWING SNAKE BITE

Snake bite is a common occupational hazard among farmers and agricultural workers in the rural areas of developing countries. Among venomous snakes, in India

viper bites are more common.[34] The two widely distributed vipers causing ARF in India are Russel's viper and Echis carinatus.[35]

The kidneys being highly vascularised organs, are highly susceptible to the venom toxins of snake. A wide range of renal manifestations have been observed which include

- Acute tubular necrosis (most common),
- Acute tubulointerstitial necrosis,
- Renal cortical necrosis,
- Vasculitis
- Mesangiolysis
- Glomerulonephritis
- Hematuria , proteinuria, myoglobinuria.[36]

AKI due to snake envenomation is mostly due to acute tubular necrosis (100%) and acute cortical necrosis (25%). Occasionally glomerular lesions (30%) are also observed.[37] Following envenomation AKI develops within one to two days.[36] Usually kidney injury following snake bite is reversible, but if acute cortical necrosis sets in, it may end up in incomplete recovery.[34]

Factors that contribute to pathogenesis of renal injury include bleeding, hypotension, circulatory collapse, disseminated intravascular coagulation, intravascular hemolysis, direct nephrotoxicity of venom, microangiopathic hemolytic anaemia, hemoglobinuria, rhabdomyolysis.[34]

Nephrotoxicity of the venom :

AKI induced by snake venom may be either due to its direct cytotoxicity on the renal structures or a secondary response of systemic envenomation triggered by inflammation, release of cytokines such as TNF-α, interleukins and vasoactive substances like nitric oxide, bradykinin, histamine, and eicosanoids. These ultimately reduce the renal blood flow and generate an ischemic process and reduced cellular oxygen delivery leading to cell injury and necrosis. Snake venom metalloproteinases, phospholipases A_2, serine proteases, hyaluronidases, esterases, L-amino oxidases and bradykinin-potentiating peptides are responsible for releasing vasoactive and inflammatory substances.[36]

Hypotension may be due to bleeding into tissues or externally, release of bradykinin, depression of medullary vasomotor centre, myocardial depression, arteriolar vasodilatation. Ultimately these factors result in ischemic ARF.

Intravascular hemolysis is mediated by phospholipase A_2 and 'direct lytic factor'. Phospholipase A_2 causes either direct hemolysis of red cell membrane or indirectly by the production of lysolecithin which is a strong hemolytic substance. Free hemoglobin levels greater than 200mg/dl in the plasma is toxic to the renal tubular cells. In the setting of hemoglobinuria, ARF sets in particularly when dehydration, hypotension or hemorrhage supervene.[35]

Disseminated intravascular coagulation :

Venom of E. carinatus directly activates the conversion of prothrombin to thrombin. Russel's viper venom induces thrombin formation by activating factor X in the presence of calcium ions and factor V.[35]

The formation of fibrin thrombi in the renal glomerular capillaries and microvasculature contributes to the renal dysfunction.

Rhabdomyolysis :

Myoglobin toxicity following rhabdomyolysis causes renal injury by following mechanisms –

- Myoglobin causes renal vasoconstriction by acting as nitric oxide scavenger by directly binding NO. This results in hypoperfusion and ischemic injury.
- Myoglobin precipitates within the renal tubules forming intraluminal obstructive casts. This precipitated myoglobin may undergo degradation to form free heme and iron, which are potential agents causing renal injury by generating ROS.

SEPSIS AND ACUTE KIDNEY INJURY

Sepsis is defined as " a severely dysregulated inflammatory response to infections characterized by dysfunction of end organs away from the primary site of infection."[38]

The hallmark of any form of sepsis is activation of the immune response system of host, as a response to exotoxins and cell wall components of the bacterial organisms.[25]

Humoral mediators of sepsis include bacterial lipopolysaccharides, microbial DNA and lipopeptides, lipoteichoic acid and peptidoglycan which trigger the

expression of toll-like receptors (especially TLR-4). This activates certain signal transduction pathways which exacerbate the generation of various cytokines.[39]

The pathogenesis of sepsis-induced AKI is multifactorial which includes hemodynamic alterations, activation of pro- as well as anti-inflammatory pathways, endothelial dysfunction, coagulation activation and immunological dysregulation, all of which culminate in multi-organ damage.[38,40]

- **Renal perfusion :** A fundamental feature of sepsis is arterial vasodilatation associated with a decrease in the systemic vascular resistance.[38] Regional redistribution of blood flow to the kidney away from the medulla, while favouring the cortex occurs during sepsis. This phenomenon is termed 'cortico-medullary redistribution'.[41]

The reversible mechanisms of sepsis-induced hypovolemia include increased vascular permeability with leakage of fluid into the interstitium, increased venous pooling and septic vasoplegia-induced hypotension.[40]

In addition, hypoxia, acidosis and mechanical ventilation, especially PEEP cause vasoconstriction. Increase in body temperature associated with sepsis leads to ATP loss and worsens the ischemic injury.[41]

Additional contributing factors include efferent arteriolar vasodilatation, peri- glomerular shunting between the afferent and efferent arteriole, neutrophil accumulation primed by endotoxin, release of vasoactive substances such as adenosine, endothelin and thromboxane A_2.[42]

- **Inflammation :** AKI following sepsis is heralded by the induction of a variety of inflammatory cytokines, thrombogenic substances, biologically active mediators and arachidonate metabolites.

The major mediators of cytokine-induced AKI are TNF and IL-1, which induce neutrophil aggregation, vasoconstriction, ROS production and thrombosis by inducing tissue factor. These cytokines also have the ability to induce mesangial and endothelial production of vasoactive substances.[43]

Sepsis induces oxidative stress. The propagation of ROS cascade is accompanied by a decrease in the endogenous ROS scavengers such as superoxide dismutase in the kidney during endotoxemia. The reactive species also scavenge nitric oxide producing peroxynitrite radicals, which add on to the renal injury. Of late, it has been found that ROS induce the endothelial secretion of multimers of von Willibrand factor and ADAMTS-13 inhibits the proteolysis vWF.[44]

- **Cellular mechanisms :**

The evolution of sepsis is characterized by dysfunction of tubular epithelial tight junctions leading to back-leak of tubular fluid into the interstitium. Tubular cast formation occurs following loss of cellular adhesion to the basement membrane.[43]

The renal tubular cells die by two mechanisms, apoptosis and necrosis. The necrotic cells release ATP which directly activate Nrlp3 inflammosome via $P2X_7$ receptor, further accelerating the tissue damage.[45]

During acute renal injury the death receptors, Fas-L and TNFR-1 are expressed which contribute to the apoptotic mechanism of cell death.

Yet another proposed mechanism of renal injury following sepsis is mitochondrial hibernation which results in an inadequate production of ATP due to a decline in the respiratory chain activity.[30]

- **Coagulation cascade :**

In response to LPS and TNF, the expression of tissue factor is induced. TF binds activated FVII, which activates FX that cleaves prothrombin to form thrombin and finally the formation of fibrin. Tissue inflammatory response is enhanced by this cascade.[43]

CONTRAST-INDUCED AKI

It presents as an acute GFR reduction by 24 to 48 hours after administration of intravenous contrast.[11] The main pathophysiological mechanisms postulated are a combination of acute renal vasoconstriction, medullary ischemia and ROS generation causing a direct renal tubular injury. A biphasic hemodynamic response is observed where an initial vasodilation is followed by sustained vasoconstriction due to vasoactive substances released from endothelial cells. This results in cortico-medullary redistribution of renal blood flow.[21,46]

RHABDOMYOLYSIS

The most common causes of rhabdomyolysis causing AKI are trauma, ischemia, drugs, metabolic abnormalities, genetic defects and infections. The spectrum of rhabdomyolysis leading to myoglobinuria and hence renal failure is related to vasoconstriction, oxidant stress causing tubular cell injury and tubular obstruction by cast formation.[2]

AKI IN PREGNANCY

Urinary tract infections which are the most common pregnancy related complications can progress to pyelonephritis. Infection following abortion can cause renal injury by sepsis, hemorrhage, hypotension and DIC.

Post-partum hemorrhage, pre-eclampsia, abruption placenta, amniotic fluid embolism are third trimester and post-partum complications which cause AKI by ischemia causing bilateral cortical necrosis.[47]

DRUG-INDUCED AKI

The factors that increase the vulnerability of kidneys to toxins are

- A wide range of drugs are excreted by the kidneys. Its concentrating mechanisms are responsible for the exposure of the renal tubular cells to intracellular accumulation of massive concentrations of drugs.

- Drugs also undergo metabolism and biotransformation by the kidney, resulting in formation of toxic compounds.

The mechanisms of drug-induced nephrotoxicity include acute tubular cell injury, intratubular obstruction, hemodynamic alterations, acute interstitial nephritis, thrombotic microangiopathy, rhabdomyolysis, hypersensitivity vasculitis and osmotic nephrosis.[48]

ACUTE INTERSTITIAL NEPHRITIS

AIN is characterized by immune – mediated tubule-interstitial injury caused by drugs, autoimmune disorders or infections.

The drug-induced hypersensitivity reactions result in AKI by inducing allergic response in the interstitium and tubules of kidney, sparing the glomeruli.

Infections caused by Legionella pneumophila, leptospira, cytomegalovirus, hepatitis B, HIV or streptococci can cause AIN by releasing chemokines, leading to infiltration of leukocytes within the interstitium.[49]

AKI IN CANCER

AKI in patients with cancer is due to

- Nephrotoxic chemotherapeutic agents such as mitomycin c, platinum compounds, gemcitabine, ifosfamide and methotrexate.
- Tumor lysis syndrome is particularly seen in patients with large tumors having a cellular turnover rate following cytotoxic therapy. Massive amounts of intracellular

contents are released into the systemic circulation, leading to accumulation of uric acid and cytoplasmic contents (potassium, phosphorus) which exceeds the excretory capacity of kidney. AKI is secondary to tubular obstruction caused by intratubular precipitation of uric acid and xanthine, resulting in acute urate nephropathy and acute nephrocalcinosis.

- In patients with multiple myeloma, AKI results due to myeloma cast nephropathy, glomerular infiltration with light chains and amyloid deposition.[50]

ACUTE PHOSPHATE NEPHROPATHY

It is a complication following preparation for colonoscopy and bowel surgery with oral phosphate solution as bowel cathartic. The pathogenesis involved is a significant increase in the serum phosphate concentration, with a simultaneous depletion of intravascular volume, which causes precipitation of calcium phosphate salts within renal tubules causing direct injury.[33]

AKI FOLLOWING CARDIAC SURGERY

The risk factors for developing post-operative AKI includes pre-operative renal function, diabetes, age, duration of cardiac bypass, blood transfusion, vascular surgery and poor cardiac function. Renal athero-embolism can result from aortic clamping and instrumentation. Activation of neutrophils, complement and fibrinolytic

systems occur following cardiac bypass. Furthermore, peri-operative left ventricular dysfunction and myocardial infarction can hamper renal perfusion.[28]

AKI IN NEPHROTIC SYNDROME

The proposed mechanisms of AKI following nephrotic syndrome include:

- Hypovolemia due to reduced plasma oncotic pressure
- Reduced GFR secondary to hypoproteinemia, exacerbated by inhibition of prostaglandin synthesis by NSAIDs
- Hypercoagulable state accompanying nephrotic syndrome predisposes to renal vein thrombosis.[51]

ORGAN CROSS TALK DURING AKI:

AKI is a systemic syndrome which can cause potentially devastating effects on other organs.

1) AKI could be associated with acute lung injury characterized by an increase in permeability of pulmonary vasculature and microvascular inflammation. Uremic toxins could result in downregulation the epithelial sodium channels of lung.[25]

2) There could also be a cross talk between kidney and bone marrow following acute ischemia characterized by an increase in the levels of Granulocyte- Colony Stimulating Factor mRNA and protein and an increase in the activity of myeloperoxidase.[25]

3) Kidneys produce IL-6 following injury which can stimulate the kupfer cells of liver to produce IL-10, augmenting further inflammatory damage to the organ.

4) Furthermore, cross talk between heart and kidney could occur, which mainly involves tumor necrosis factor–α, IL-1, ICAM-1 mRNA and apoptotic mechanisms.[21]

CONSEQUENCES & COMPLICATIONS OF AKI

Consequences include significant risk of developing chronic kidney disease especially stage 4 CKD, long term dialysis dependence, increased mortality, and personal and community costs.[10, 30]

Complications include :

1)**Metabolic complications** : hyperkalemia, metabolic acidosis, hyponatremia, hypocalcemia, hyperphosphatemia, hypermagnesemia, hyperuricemia, uremia.

2)**Cardiovascular complications** : pulmonary edema, arrhythmias, pericarditis, pericardial effusion, pulmonary embolism, hypertension, myocardial infarction.

3)**Gastro-intestinal complications**: nausea, vomiting, gastrointestinal hemorrhage.

4)**Neurological complications** : neuromuscular irritability, asterixis, seizures.

5)**Hematological complications** : bleeding and anemia due to inhibition of erythropoiesis, hemolysis, hemodilution and reduced RBC survival time.

5)**Infections** : pneumonia, septicemia, urinary tract infections.

7)**Malnutrition** due to loss of appetite, increased catabolism and nutrient losses in dialysate.

8)Other complications: hiccups, elevated parathormone, low total T_3 and T_4.[21,25]

KIDNEY INJURY MOLECULE - I

Kidney injury molecule-1 (KIM-1) is a transmembrane renal tubular protein whose expression is markedly induced in acute as well as chronic renal disease. The cDNA for this membrane protein was first discovered by Ichimura et al, from post-ischemic kidney of rat.[4]

KIM-1 is one of the members of immunoglobulin gene superfamily (IgSF), which is structurally most closely identical to 'mucosal addressin cell adhesion molecule-1' (MAdCAM-1)[52]

STRUCTURE OF KIM-1

KIM-1 is a type-1 membrane glycoprotein with a molecular weight of 104kDa and consists of

- An extracellular part, containing a novel six-cysteine immunoglobulin-like domain, two putative sites of N-glycosylation and a mucin domain which is rich in Threonine/Serine-Proline, where O-glycosylation of the polypeptide chain can occur.[3]
- A transmembrane domain, and
- A cytoplasmic domain which is relatively short and contains a highly conserved tyrosine kinase phosphorylation site which is phosphorylated by tyrosine. This indicates that KIM-1 could act as a signaling molecule.[4,53]

The Ig-like domains mediate cell surface protein interactions which are responsible for interactions between cell to cell and cell to matrix.[32]

Jentoft proposed the “lollipop on a stick” model which states that the mucin domain functions as a configurational domain which extends the Ig-like domain sufficiently above the plasma membrane, like a stalk. Mucins could be involved in cell adhesion and also protect the epithelial cellular surface.[32,52]

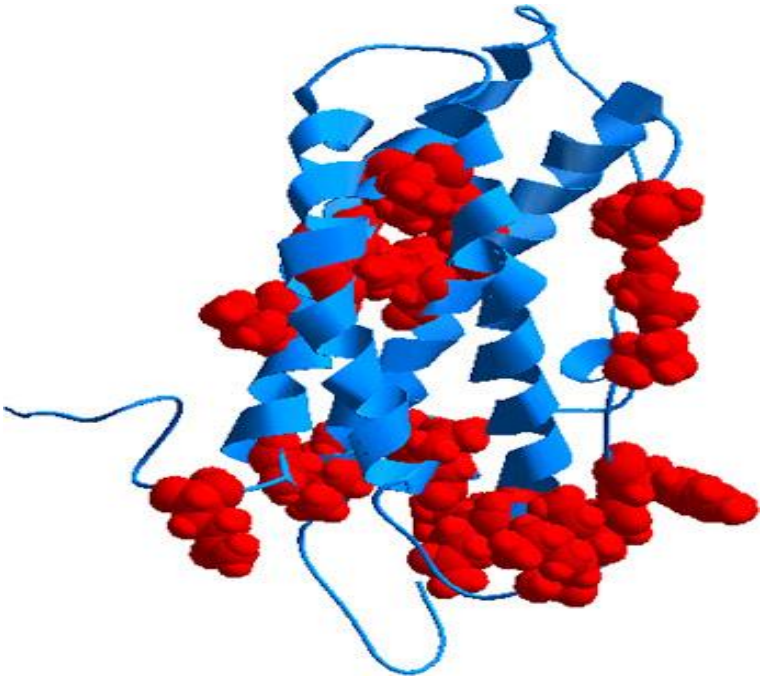
Molecular Structure of KIM-1

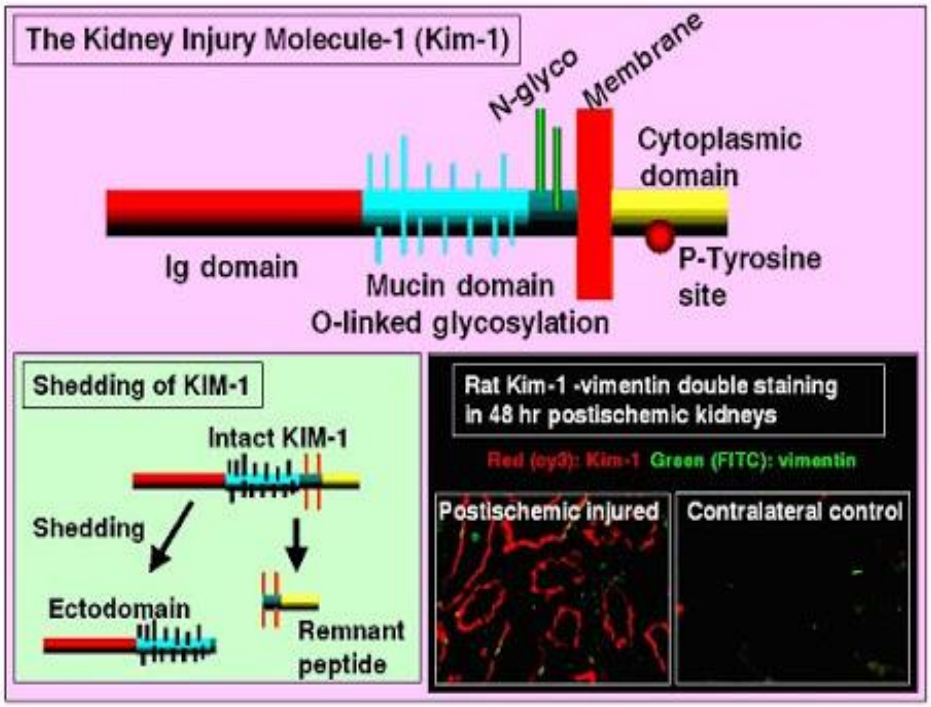
The open reading frame of KIM-1 consists of 307 amino acids. The signal sequence is of 21 amino acids, the trans-membrane spanning domain of 23 amino acids from 235 to 257 and the cytoplasmic portion consists of 50 amino acids.

The extracellular domain consists of 213 amino acids. It contains two distinct domains. Within its amino terminal is a region which is homologous to the variable regions of IgSF with respect to two cysteine residues (Cys^{37} and Cys^{108}). In addition to these two residues, KIM stands unique due to the presence of four additional cysteine residues in its Ig domain. The mucin domain is from amino acids 131-201. [52]

The two putative N-glycosylation sites are found between the mucin domain and transmembrane domain.[52]

Figure 3 : Structure of KIM-1





TYPES OF KIM-1

KIM-1 was first discovered in African green monkeys as the cellular receptor for hepatitis-A virus (HAVcr-1). Subsequently two homologs of KIM-1 were cloned in humans.

a) KIM-1a (hepatic form) : cloned from liver as the homolog of KIM-1
b) KIM-1b (renal form) : cloned from kidney as the homolog of HAVcr-1

These two homologs are splice variants which differ in their C-terminal part of cytoplasmic domain.[54]

KIM-1 was recently discovered to be expressed also in activated Th-1 and Th-2 lymphocytes. Hence it is othervise called as Tim-1 (T-cell immunoglobulin and mucin-containing molecule). Tim-1 is a member of closely related molecules, with 8 types found in mice (Tims1-8) and 3 in humans (Tims1, 3 and 4). Tim-1 is mainly expressed in Th-2 cells and involved in airway hyper-responsiveness and atopic disease, whereas Tim-4 in dendritic cells and macrophages. They act as co-stimulating molecules positive regulators of activity of T-cells.[54,55]

SHEDDING OF THE ECTODOMAIN:

KIM-1 is dramatically upregulated in dedifferentiated epithelium of proximal tubules of the kidney, as an early response to injury.[53] Subsequently, the heavily glycosylated ectodomain of KIM-1 is shed from the surface of the cells into the tubular lumen.[3] This surface proteolytic cleavage of the transmembrane glycoprotein, leads to

release of a soluble form of 90kDa into the extracellular matrix , leaving a C-terminal stalk associated with the cell.[32]

This process is found to be regulated by mitogen activated protein kinase (MAPK) signaling pathway, which is induced by cell stress.[4] The enzymes that participate in cleavage are matrix metalloproteinases (MMPs) or members of the ADAM family (a desintegrin and metalloproteinase).[32]

The ectodomain that is shed in the urine has been found to be sufficiently stable for a prolonged period of time.[56] This adds to the value of KIM-1 as a biomarker because the expression of KIM-1 in the tissue is closely correlated with its excretion in the urine.[54]

The function of the ectodomain is that it interacts with integrins in the apical membrane of tubular cells, restraining their depolarization. This prevents the exfoliated cells from getting attached to one another, producing tubular obstruction by forming casts.[4]

KIM-1 IN KIDNEY INJURY

KIM-1 has been discovered as the most highly expressed proximal tubular protein in response to renal injury. Following an acute insult, KIM-1 mRNA rapidly translates to generate massive amounts of the protein which localizes on the proximal tubular apical membrane.

In humans following ischemia or toxic renal injury, all three segments of proximal tubule show increased KIM-1 expression, although it is most prominently

expressed in the S_3 segment, being most susceptible to injury due to ischemia or toxins.[4,57] The expression of KIM-1 gene in the kidney correlates with the expression of KIM-1 protein in the kidney as well as urine.[58]

The concentration of KIM-1 in the urine of healthy humans is less than 1ng/ml.

FUNCTIONAL ROLE OF KIM-1

a) **Role as a scavenger receptor :** KIM-1 has been established as a functional phosphatidyl serine receptor which confers the properties of phagocytosis on the epithelial cells in the post-ischemic kidney. KIM-1 ctransforming the epithelial cells of the tubule into semiprofessional phagocytes. This involves not only binding to the cell surface, but also triggers internalization.[26] These transformed cells clear off the necrotic and apoptotic cell debris from the tubular lumen, which is critical for remodeling following injury, hence relieving intratubular obstruction.[4]

KIM-1 has been found to be the first epithelial cell scavenger receptor which is found in non-myeloid cells.[3,26] KIM-1 also has the properties of macrophage scavenger receptor type B that binds oxidized LDL.[57]

b) **Anti-inflammatory role :** KIM-1 has an anti-inflammatory role by mediating phagocytosis. This protective effect is mucin domain-dependant.

KIM-1 interacts with p85 and subsequently PI3K mediates the downregulation of NFkB. This culminates in a decline in the TLR-4 expression, reduced pro-inflammatory

cytokines and macrophage activity. This protects the kidney by dampening inflammation and innate immunity.[59]

c) **Role in immunity :** In immune system, KIM-1/TIM-1 mediates the activation of Th1, Th2 and Th17 differentiation by acting as surface receptor on T-cell, interacting with antigen presenting cells. It also functions as a receptor which activates the natural killer T-cells, dendritic cells and B-cells.[5,60]

d) **Adhesion molecule :** KIM-1 has many roles in epithelial function. It interconnects the cells to one another and also tethers the epithelial cells to the extracellular matrix.[4]

e) **Role in regeneration and repair :** Following an acute insult, KIM-1 plays an important role in maintaining morphological and functional integrity of the kidney.[54] The surviving epithelial cells undergo cell locomotion, proliferation and dedifferentiation mediated by KIM-1 which is necessary for regeneration and repair of the injured epithelium.[31] KIM-1 is involved in the migration of dedifferentiated cells, hence facilitating the reconstitution of continuity of epithelial layer.[4,32]

AIMS & OBJECTIVES

AIM OF THE STUDY

1. To estimate urinary KIM-1 level in cases of acute kidney injury.
2. To compare the level of urinary KIM-1 with serum creatinine and blood urea and to prove the utility of urinary KIM-1 as an early biomarker of acute kidney injury.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College, Thanjavur, after getting approval from the Ethical Committee of Thanjavur Medical College, Thanjavur.

The study is a cross-sectional and analytical study which was conducted from January 2014 to September 2015. A total of 100 subjects were chosen. Both males and females in the age group above 18 years were included and an informed consent was obtained from all of them. The study population included two groups.

One group consists of 50 patients who were admitted for snake bite, and the other group consists of 50 patients who were admitted with sepsis. The patients who did not develop AKI were considered as controls.

INCLUSION CRITERIA :

- Patients admitted within 12 hours of snake bite.
- Patients who developed sepsis with the presence of a septic focus.
- Age > 18 years

EXCLUSION CRITERIA :

- Chronic kidney disease
- Snake bite and sepsis patients with elevated serum creatinine on admission

- Known hypertensive on treatment
- History of diabetes mellitus
- History of Polycystic Kidney Disease
- Nephrotic syndrome

STUDY PROTOCOL

A detailed history was elicited for

- Co-morbid diseases and concomitant drug intake
- History of snake bite, time and site of bite, species of snake, native treatment, treatment before hospitalization.
- History of reduced urine output
- History of infections, fever.

Clinical examination:

A thorough physical examination was done to look for local and systemic features of envenomation like fang marks, cellulitis, bleeding from the site of bite, local necrosis, blistering, gangrene, regional lymph node enlargement and features of gum bleeding, epistaxis, ecchymosis.

A systemic examination was done to assess for the presence of respiratory, genitourinary, gastrointestinal, central nervous system or musculoskeletal infections.

All vital signs were looked for. Features of uremic symptoms were looked for.

SAMPLE COLLECTION

Urine :

Urine samples were collected in sterile tubes. The samples were centrifuged at 2000 rpm for about 20 minutes. Supernatant was carefully collected and stored by freezing at -20°C. When sediments occurred during storage, again centrifugation was done.

Serum :

The serum was allowed to clot for 20 minutes at room temperature. Then the samples were centrifuged at 2000rpm for 20 minutes. Supernatant was collected carefully and stored at -20°C.

INVESTIGATIONS :

1. Urinary KIM-1
2. Serum creatinine
3. Serum urea
4. Complete blood count
5. Clotting time

ESTIMATION OF URINARY KIDNEY INJURY MOECULE – 1

METHODOLOGY :

enzyme-linked immune sorbent assay (ELISA)

PRINCIPLE & PROCEDURE:

The basis of ELISA used by this kit is Biotin double antibody sandwich technology to assay human KIM-1. Samples and calibrators containing KIM-1 were added to the wells that are pre-coated with KIM-1 monoclonal antibody and then incubated. Then biotin-labelled anti-KIM-1 antibody was added which unites with strptavidin-HRP forming immune complexes. The unbound enzymes were removed after incubation by washing. Substrates A and B were added which changes the colour of the solution to blue and then yellow by the effect of acid. The shades of solution positively correlate with the concentration of Human KIM-1.

MATERIALS SUPPLIED IN THE KIT:

1. Standard solution (12.8ng/ml) - 0.5ml
2. Standard dilution - 3ml
3. Coated ELISA plate - 12 wells × 8 tubes
4. Streptavidin-HRP - 6ml
5. Washing concentration (30X) - 20ml

6. Anti-KIM-1 antibodies labeled with biotin - 1ml
7. Chromogen solution A - 6ml
8. Chromogen solution B - 6ml
9. Stop solution - 6ml

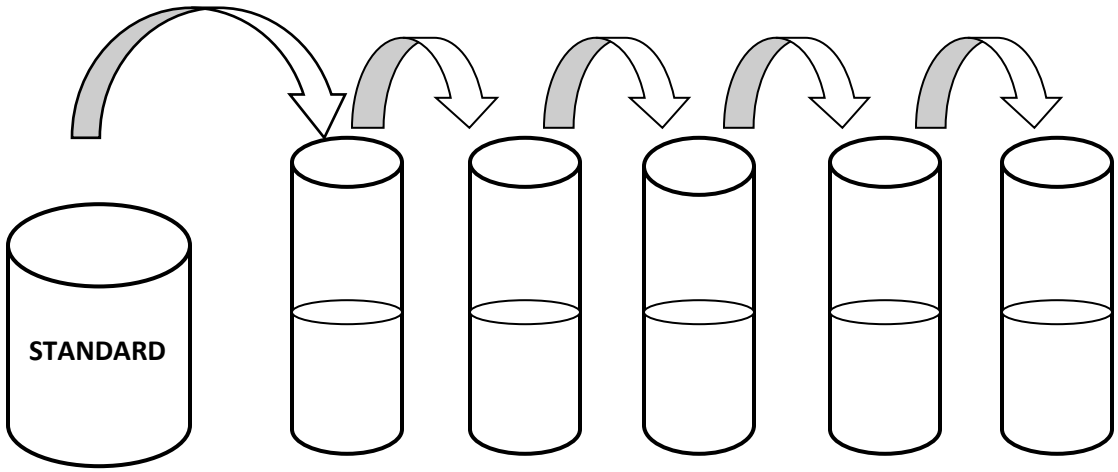
Other materials required were

10. 37°C incubator
11. Precision pipettes and disposable pipette tips
12. Disposable tubes for sample dilution
13. Standard enzyme reader
14. Distilled water
15. Adsorbent paper

PREPARATION OF REAGENTS

- All samples and reagents were brought to room temperature which took about 30 minutes. Specimens were mixed thoroughly by gentle inversion and visible particulate matter was cleared by low speed centrifugation.
- Wash solution : 30X wash solution was diluted by pouring the total contents of the bottle (20ml) into a 1L graduated cylinder and 580ml of deionised water was added to make a final volume of 600ml. It was then thoroughly mixed.
- Dilution of standard solution : The kit had a standard of original concentration which was supposed to be diluted in small tubes by following the instructions:

Standard No.5	6.4ng/ml	120µl original standard + 120µl standard diluent
Standard No.4	3.2 ng/ml	120µl Standard No.5 + 120µl standard diluent
Standard No.3	1.6 ng/ml	120µl Standard No.4 + 120µl standard diluent
Standard No.2	0.8 ng/ml	120µl Standard No.3 + 120µl standard diluent
Standard No.1	0.4 ng/ml	120µl Standard No.2 + 120µl standard diluent



12.8 ng/ml 6.4ng/ml 3.2ng/ml 1.6ng/ml 0.8 ng/ml 0.4 ng/ml

- Streptavidin-HRP : ready to use
- Biotin labeled Anti KIM-1 antibodies : ready to use
- Chromogen solutions A and B : ready to use
- Stop solution : ready to use

ASSAY PROCEDURE

- The number of stripes needed was decided by the number of samples to be tested and by that of standards.
- Sample injection :
    1. Blank well : no sample, anti KIM-1 antibody or streptavidin-HRP was added to the blank well. Chromogen solutions A and B, and stop solution were added.
    2. Standard solution well : 50µl standard and 50µl streptavidin-HRP were added. Since the biotin antibodies are already united in the standard, they need not be added.
    3. Sample well : 40µl sample, 10µl biotin labeled KIM-1 antibodies and then 50µl streptavidin-HRP were added. Then the ELISA plate was covered with a seal plate membrane.

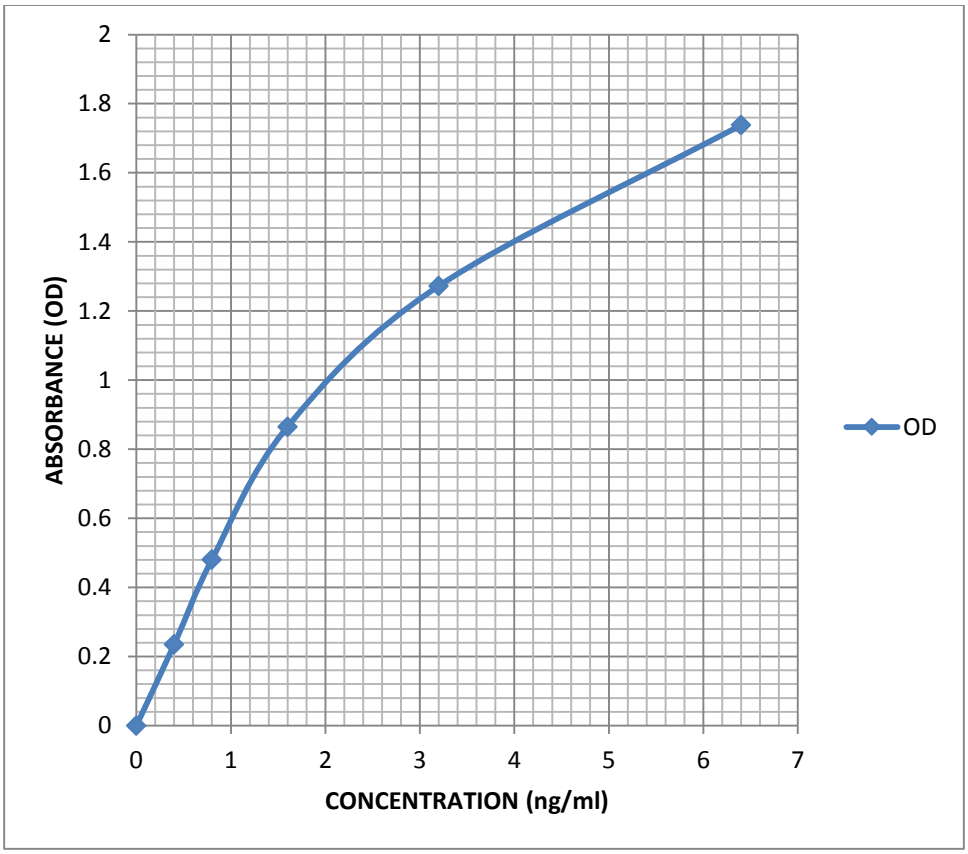
The plate was shaken gently to mix them up and then it was incubated at 37°C for 60 minutes.

- Washing : The seal plate membrane was carefully removed, liquid drained and the remaining liquid shaken off. Each well was filled with 350µl wash solution. After standing for 30 seconds, the liquid was drained. To blot the ELISA plate, it was pat hard on bibulous papers on the test bed, several times downward. The procedure was repeated 5 times.

- Color development : First, 50µl chromogen solution A was added to each well, and then 50µl chromogen solution B was added to each well. They were mixed by gentle shaking. Incubation was done for 10 minutes at 37°C away from light for the color to develop.
- Stop : To stop the solution 50µl stop solution was added to each well. At this moment, there was an immediate change in color from blue to yellow.
- Assay : Taking blank as zero, the absorbance (OD) of each well was measured at 450nm wavelength within 10 minutes after adding the stop solution.
- According to the concentration of standards and their corresponding OD values, the linear regression equation of standard curve was calculated. Then the concentration of the sample was calculated according to their corresponding OD values.

CALCULATION OF RESULTS

Calibration graph : The standard curve was constructed by plotting concentration of each KIM-1 calibrator in ng/ml along the x-axis against the OD values of the corresponding calibrator along y-axis.



KIM-1 values of samples :

The KIM-1 concentration of each sample was found by locating the point on the curve corresponding to the absorbance values for the samples and reading its corresponding concentration in ng/ml from the x-axis.

ASSAY RANGE : 0.5ng/ml - 10ng/ml

SENSITIVITY : 0.01ng/ml

VALIDITY & STORAGE : 6 months when stored at 2-8°C and 12months at -20°C

QUANTITATIVE ESTIMATION OF SERUM CREATININE

MODIFIED JAFFE'S REACTION, INITIAL RATE

PRINCIPLE OF THE METHOD

Creatinine reacts with picric acid in an alkaline medium to form an orange-yellow color which is termed as Jaffe's reaction. The initial rate method is introduced to improve the specificity of the test. The optical density of the orange-yellow color formed is directly proportional to the concentration of creatinine, which is measured photometrically at 500 to 520nm.

COMPOSITION OF THE REAGENT

- Reagent 1 - Picric Acid Reagent

  Picric acid : 25.8 mmol/L

- Reagent 2 - Sodium Hydroxide Reagent

  Sodium hydroxide : 95 mmol/L

- Creatinine standard

  Creatinine standard : 2 mg/dl (0.166 mmol/L)

REAGENT PREPARATION

Equal volumes of Reagent 1 and Reagent 2 are mixed and wait for 15 minutes before use.

STORAGE AND STABILITY

Reagents 1, 2 and standard when unopened remain stable till the expiry date. The Working Reagent is stable for 21 days at 2-8°C. The absorbance of the reagent blank should be <0.3 at 505nm when read against distilled water.

ASSAY PARAMETERS

Wavelength (nm) : 505

Mode : fixed time

Sample volume : 100 µl

Reagent volume : 1000 µl

Lag time : 20 sec

Kinetic interval : 60 sec

No. of readings : 1

Reaction temperature : 37°C

Normal low : 0.6 mg/dl

Normal high : 1.4 mg/dl

Reaction direction : increasing

Linearity low : 0 mg/dl

Linearity high : 25 mg/dl

Absorbance limit (max) : 0.3

Standard concentration : 2 mg/dl

Blank with : water

Units : mg/dl

ASSAY PROCEDURE

PIPETTE	STANDARD	TEST
Working reagent	1000µl	1000µl
Standard	100µl	-
Test	-	100µl

The test tubes were shaken to mix the contents and the initial absorbance was read within 20 seconds of mixing and final absorbance at 80 seconds.

CALCULATION

The results are calculated as follows :

$\Delta A = A_2 - A_1$

$$\text{Creatinine concentration in mg/dl} = \frac{\Delta A \text{ of the test}}{\Delta A \text{ of standard}} \times \text{concentration of standard(mg/dl)}$$

LINEARITY

The assay is linear upto a value of 25mg/dl. For higher values the samples were diluted with normal saline and the assay repeated. Then the results were multiplied with the dilution factor.

NORMAL VALUES

For males : 0.7 - 1.4 mg/dl

For females : 0.6 - 1.2 mg/dl

QUANTITATIVE DETERMINATION OF UREA BY UREASE METHOD (GLUTAMATE DEHYDROGENASE - FIXED TIME)

The method is used to determine urea in serum / plasma.

METHODOLOGY

It is a kinetic, enzymatic method.

PRINCIPLE OF THE METHOD

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \text{2-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of change of absorbance at 340nm is directly proportional to the concentration of urea in serum.

REAGENT COMPOSITION

Tris buffer (pH 7.8) : 96 mmol/L

ADP : 0.6 mmol/L

Urease : 16000U/L

GLDH : 960 U/L

NADH : 0.25 mmol/L

2-Oxoglutarate : 9 mmol/L

Urea standard : 50 mg/dl

WORKING REAGENT PREPARATION & STABILITY

Working reagent is prepared by mixing 4 parts of reagent R1 with one part of reagent R2.

The working reagent (4R1 : 1R2) is stable for 30 days at 2-8°C, when protected from light and contamination. Fresh working solution is prepared before the assay is performed.

STORAGE & STABILITY

Reagent solutions R1 and R2 and standard are stable when unopened, till the expiry date when stored at 2-8°C.

Reagent deterioration may be detected when there is turbidity or when the reagent blank absorbance is <0.8 at 340nm.

ASSAY PARAMETERS

Mode : fixed time

Wavelength : 340 nm

Sample volume : 20 µl

Reagent volume : 1000 µl

Lag time : 20 seconds

Kinetic interval : 60 seconds

No. of readings : 1

Reaction temperature : 37°C

Reaction direction : decreasing

Normal low : 13 mg/dl

Normal high : 45 mg/dl

Linearity low : 0 mg/dl

Linearity high : 250 mg/dl

Absorbance limit (min.) : 0.8

Blank with : water

Concentration of standard: 50 mg/dl

Units : mg/dl

ASSAY PROCEDURE

PIPETTE	STANDARD	TEST
Working reagent	1000 µl	1000 µl
Standard	20 µl	-
Sample	-	20 µl

CALCULATION

$$\text{Urea concentration in mg/dl} = \frac{\Delta \text{ Absorbance of the test}}{\Delta \text{ Absorbance of standard}} \times \text{concentration of standard(mg/dl)}$$

REFERENCE VALUES

In serum/plasma : 13 - 45 mg/dl

LINEARITY

The assay is linear upto 250 mg/dl. . For higher values the samples were diluted with normal saline and the assay repeated. Then the results were multiplied with the dilution factor.

SENSITIVITY : 2.0 mg/dl

INTERFERENCE:

Haemoglobin interferes upto 400 mg/dl, ascorbate upto 30 mg/dl, bilirubin upto 30 mg/dl and triglycerides upto 2000 mg/dl do not interfere with the test.

RESULTS & STATISTICS

STATISTICAL ANALYSIS

- Student's t-test was employed for the statistical analysis of data.
- The data were expressed in terms of mean and standard deviation.
- 'P' value less than 0.05 was taken as the significant value.
- Correlation between the measured parameters was assessed using Pearson's correlation coefficient.

RESULTS

A total of 100 subjects were selected for the study. This included 50 patients with snake bite and 50 patients with sepsis.

Levels of urinary KIM-1 was estimated on the day of admission within 24 hours. Serum creatinine and blood urea were estimated on the day of admission and on the third day for all the samples. Those patients who developed AKI were considered as cases and those who did not develop AKI were considered as controls. The stage of AKI was interpreted using RIFLE criteria.

The values obtained in snake bite and sepsis cases are presented in the master charts I and II respectively.

Table 1 :

Shows comparison of urinary levels of KIM-1 in cases and controls with snake bite.

The mean value of urinary KIM-1 in cases was 4.812 ng/ml and this was significantly higher than that of control group (0.544 ng/ml, ‘p’ < 0.001).

Table 2 :

Shows age matched urinary KIM-1 values between cases and controls with snake bite.

There is no significant difference in urinary KIM-1 values in different age groups in different age groups among cases with ‘p’ value of 0.392 and among controls with ‘p’ value of 0.652.

Table 3 :

Shows urinary KIM-1 values among males and females in cases and controls with snake bite.

The mean level in males (0.54ng/ml) and females (0.55ng/ml) among controls and males (5.81ng/ml) and females (2.9ng/ml) among cases was compared. There was significant difference in urinary KIM-1 values between males and females among cases with ‘p’ value of 0.001, whereas there was no significant gender related difference among control group (‘p’ – 0.906).

Table 4 :

Shows comparison of serum creatinine values on day 1 and day 3 among cases and controls with snake bite.

There is no significant change in serum creatinine level on day 1 between cases (mean : 0.864mg/dl) and controls (mean : 0.808mg/dl) with a ‘p’ value of 0.3557. On day 3, serum creatinine is significantly elevated in cases (mean : 3.152mg/dl) than in controls (mean : 1.03mg/dl) with a ‘p’ value of < 0.001.

Table 5 :

Shows comparison of blood urea values on day1 and day 3 among cases and controls with snake bite.

Blood urea is not significantly elevated on day 1 between cases (mean: 33.64mg/dl) and controls (mean : 32.52mg/dl) with a ‘p’ value of 0.607. On day 3, blood urea is significantly elevated in cases (mean: 86.68mg/dl) than controls (mean: 40.6mg/dl) with a ‘p’ value of < 0.001.

Table 6 :

Shows comparison of urinary KIM-1, serum creatinine and blood urea between cases and controls with snake bite on day 1.

There was a significant rise in the levels of urinary KIM-1 among cases than controls with a ‘p’ value of < 0.001 on day 1. On the other hand there was no significant rise in levels of serum creatinine (‘p’ value 0.356) and blood urea (‘p’ value 0.607) between cases and controls on day 1.

Table 7 :

Shows the Pearsons correlation coefficient of urinary KIM-1 with serum creatinine and blood urea on day 1 and day 3 among cases with snake bite.

There was no significant correlation of urinary KIM-1 with serum creatinine and blood urea on day1. But on day 3, there was a significant positive

correlation of urinary KIM-1 with serum creatinine and blood urea among cases with snake bite.

Table 8 :

Shows comparison of urinary levels of KIM-1 in cases and controls with sepsis.

The mean value of urinary KIM-1 in cases was 4.544 ng/ml and this was significantly higher than that of control group (0.56 ng/ml, 'p' < 0.001).

Table 9 :

Shows age matched urinary KIM-1 values between cases and controls with sepsis.

There is no significant difference in urinary KIM-1 values in different age groups among cases with a 'p' value of 0.3 and among controls with a 'p' value of 0.754.

Table 10 :

Shows urinary KIM-1 values among males and female groups in cases and controls with sepsis.

The mean level in males (0.557ng/ml) and females (0.564ng/ml) among controls and males (4.306ng/ml) and females (5.05ng/ml) among cases was compared. There was no significant difference in urinary KIM-1 values between males and females among cases with 'p' value of 0.944, and among control group ('p' value 0.449).

Table 11 :

Shows comparison of serum creatinine values on day 1 and day 3 among cases and controls with sepsis.

There is no significant change in serum creatinine level on day 1 between cases (mean : 1.072mg/dl) and controls (mean : 0.86mg/dl) with a ‘p’ value of 0.056. On day 3, serum creatinine is significantly elevated in cases (mean : 3.656mg/dl) than in controls (mean : 1.034mg/dl) with a ‘p’ value of < 0.001.

Table 12 :

Shows comparison of blood urea values on day 1 and day 3 among cases and controls with sepsis

Blood urea is not significantly elevated on day 1 between cases (mean : 33.44mg/dl) and controls (mean : 32 mg/dl) with a ‘p’ value of 0.667. On day 3, blood urea is elevated in cases (mean : 102.76mg/dl) than controls (mean : 38.52mg/dl) with a ‘p’ value of < 0.001.

Table 13 :

Shows comparison of urinary KIM-1, serum creatinine and blood urea between cases and controls with sepsis on day 1.

There was a significant rise in the levels of urinary KIM-1 among cases than controls with a ‘p’ value of < 0.001 on day 1. On the other hand there was no

significant rise in levels of serum creatinine (‘p’ value 0.56) and blood urea (‘p’ value 0.667) between cases and controls on day 1.

Table 14 :

Shows the Pearsons correlation coefficient of urinary KIM-1 with serum creatinine and blood urea on day 1 and day 3 among cases with sepsis.

There was no significant correlation of urinary KIM-1 with serum creatinine, however on day 3, there was a significant positive correlation of urinary KIM-1 with serum creatinine.

There was a significant positive correlation of urinary KIM-1 with blood urea on day 1. A more significant positive correlation was observed on day 3 between urinary KIM-1 and blood urea among cases with sepsis.

Table 15 :

Shows the distribution of snake bite cases who developed AKI according to KDIGO staging of AKI and urinary KIM-1 levels.

Table 16 :

Shows ANOVA test which was applied to test the difference in mean KIM-1 levels among groups with different stages of AKI following snake bite.

There was significant difference (‘p’<0.001) in urinary KIM-1 levels among groups with different stages of AKI.

Table 17 :

Shows the distribution of sepsis cases who developed AKI according to KDIGO staging of AKI and urinary KIM-1 levels.

Table 18:

Shows ANOVA test which was applied to test the difference in mean KIM-1 levels among groups with different stages of AKI following sepsis.

There was significant difference (‘p’<0.001) in urinary KIM-1 levels among groups with different stages of AKI.

Figure 4 :

Bar diagram showing comparison of urinary KIM-1 values among cases and controls with snake bite.

Figure 5:

Bar diagram showing urinary KIM-1 in different age groups among cases and controls with snake bite.

Figure 6 :

Bar diagram showing comparison of urinary KIM-1 in males and females among cases and controls with snake bite.

Figure 7 :

Bar diagram showing comparison of serum creatinine on day 1 and day 3 among cases and controls with snake bite.

Figure 8 :

Bar diagram showing comparison of blood urea on day 1 and day 3 among cases and controls with snke bite.

Figure 9 :

Bar diagram showing comparison of urinary KIM-1 among cases and controls with sepsis.

Figure 10 :

Bar diagram showing urinary KIM-1 in different age groups among cases and controls with sepsis.

Figure 11 :

Bar diagram showing urinary KIM-1 in males and females among cases and controls with sepsis.

Figure 12 :

Bar diagram showing comparison of serum creatinine on day 1 and day 3 among cases and controls with sepsis.

Figure 13 :

Bar diagram showing comparison of blood urea on day 1 and day 3 among cases and controls with sepsis.

Figure 14 :

Box plot showing comparison of urinary KIM-1 among 3 stages of AKI in snake bite cases.

Figure 15 :

Box plot showing comparison of urinary KIM-1 among 3 stages of AKI in sepsis cases.

TABLE 1 - Comparison of urinary KIM-1 among cases and controls with snake bite

KIM-1 values (ng/ml)	Control	Cases
Range	0.1 - 0.9	1.2 - 8.9
Mean	0.544	4.812
Standard deviation	0.24	2.53
'p' value	<0.001 Significant	

FIGURE 4

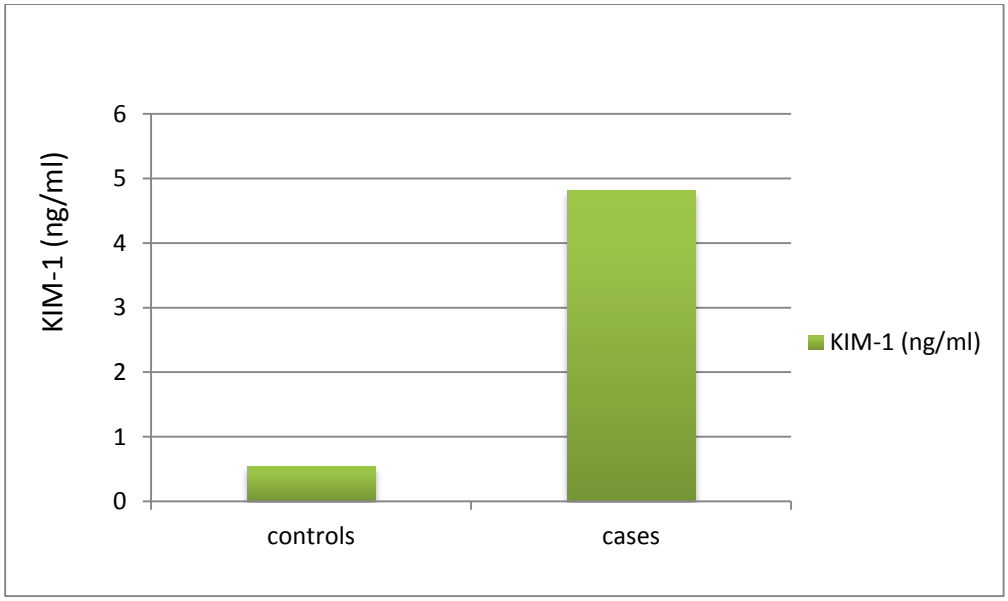


TABLE 2 - Comparison of urinary KIM-1 in different age groups among cases and controls with snake bite

Age group	Controls		Cases	
	Mean	S.D.	Mean	S.D.
<40 years	0.523	0.283	4.44	2.438
≥40 years	0.567	0.187	5.37	2.697
'p' value	0.652 Not significant		0.392 Not significant	

FIGURE 5

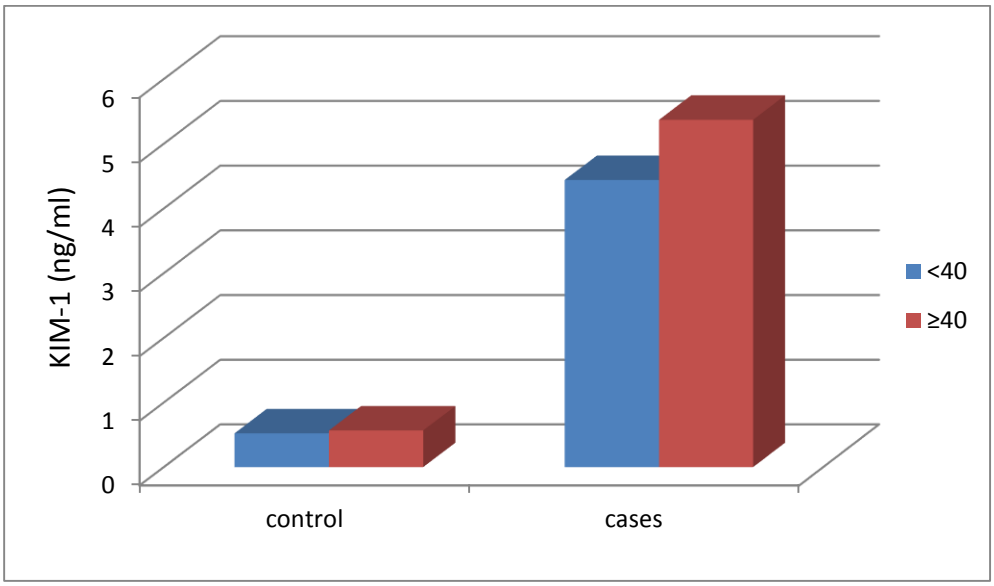


TABLE 3 - Comparison of urinary KIM-1 among males and females in cases and controls with snake bite

Sex	Controls		Cases	
	Mean	S.D.	Mean	S.D.
Males	0.538	0.272	5.81	2.37
Females	0.55	0.206	2.9	1.238
'p' value	0.906 Not significant		0.001 Significant	

FIGURE 6

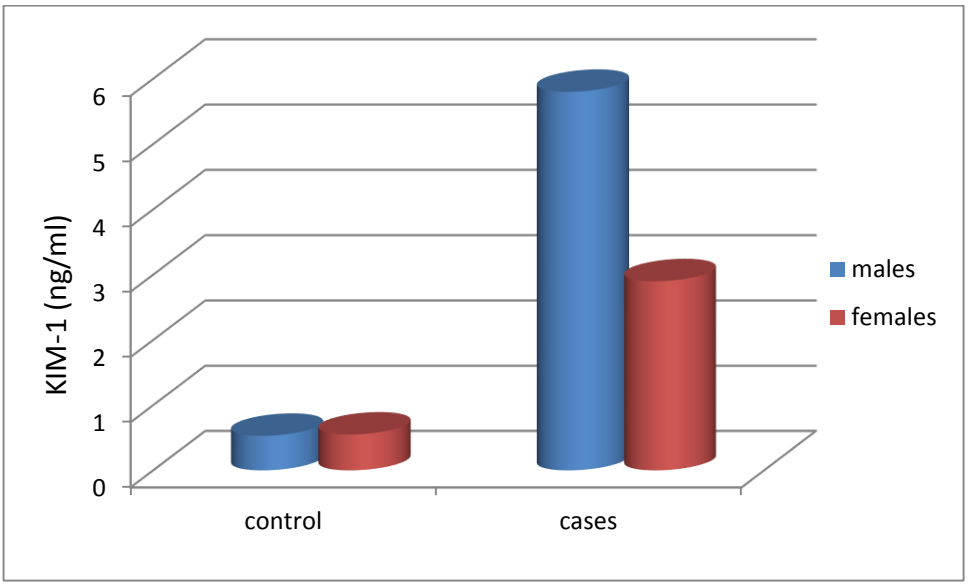


TABLE 4 - Comparison of serum creatinine on day 1 and day 3 among cases and controls with snake bite

Creatinine values on	Controls		Cases		'p' value
	Mean	S.D.	Mean	S.D.	
Day 1	0.808	0.191	0.864	0.175	0.3557 Not significant
Day 3	1.03	0.232	3.152	2.24	0.0001 Significant

FIGURE 7

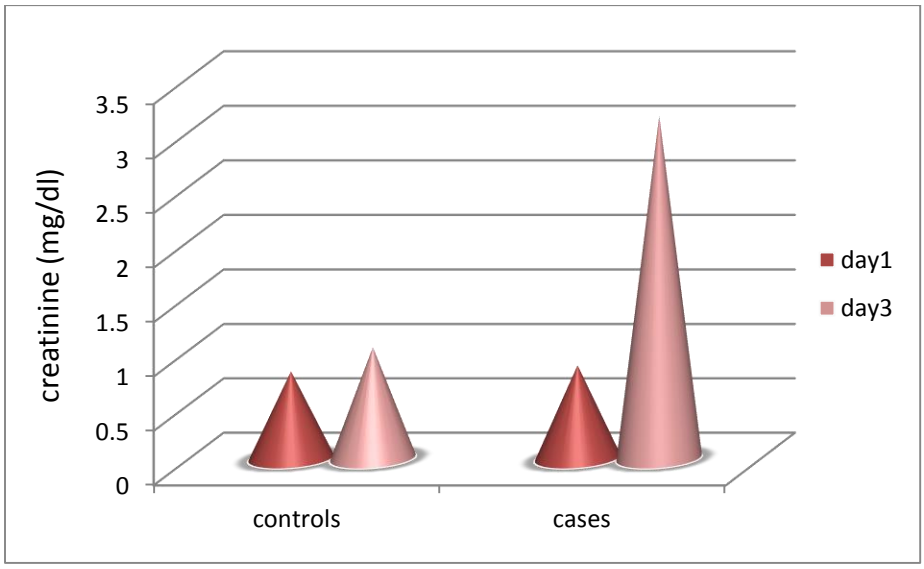


TABLE 5 - Comparison of blood urea on day 1 and day 3 among cases and controls with snake bite

Urea values on	Controls		Cases		'p' value
	Mean	S.D.	Mean	S.D.	
Day 1	32.52	6.66	33.64	7.28	0.607 Not significant
Day 3	40.06	9.87	86.68	40.40	<0.001 Significant

FIGURE 8

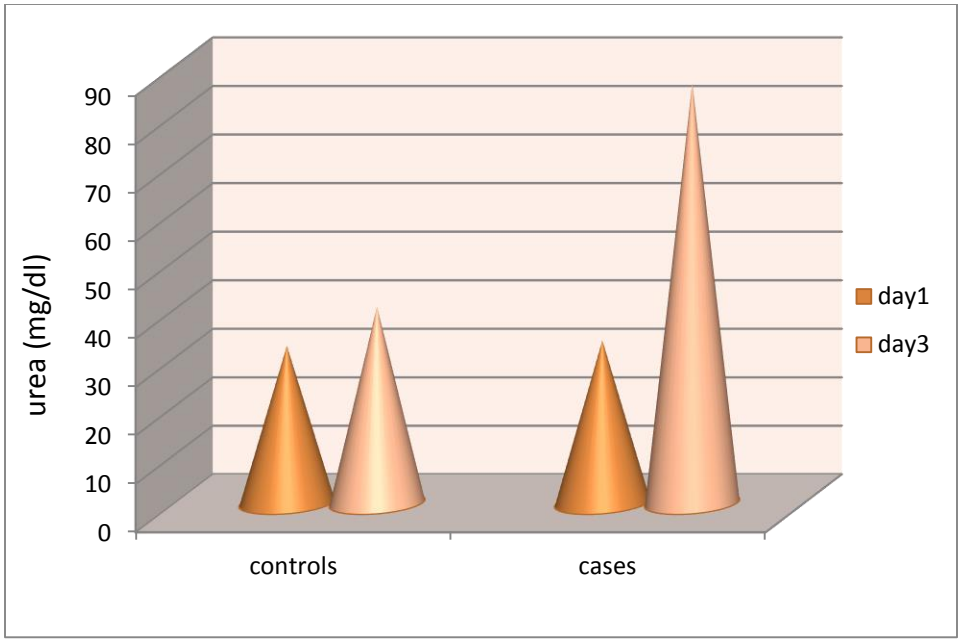


TABLE 6 - Comparison of urinary KIM-1 with serum creatinine and blood urea on day 1 in patients with snake bite

Day -1		Mean	S.D.	'p' value
KIM-1	Cases	4.812	2.53	<0.001 Significant
	Controls	0.544	0.24	
Serum creatinine	Cases	0.864	0.175	0.356 Not significant
	Controls	0.808	0.191	
Blood urea	Cases	33.64	7.28	0.607 Not significant
	Controls	32.52	6.66	

TABLE 7 - Pearson's correlation coefficient between urinary KIM-1, serum creatinine and blood urea in patients with snake bite

Correlation between	Correlation coefficient	Correlation
KIM-1 and creatinine on day 1	0.094	Not correlated
KIM-1 and creatinine on day 3	0.882	**Correlated**
KIM-1 and urea on day 1	0.380	Not correlated
KIM-1 and urea on day 3	0.864	**Correlated**

TABLE 8 - Comparison of urinary KIM-1 among cases and control with sepsis

KIM-1 values	Control	Cases
Range	0.2 - 0.9	1.2 - 7.9
Mean	0.56	4.544
Standard deviation	0.216	2.268
'p' value	<0.001 Significant	

FIGURE 9

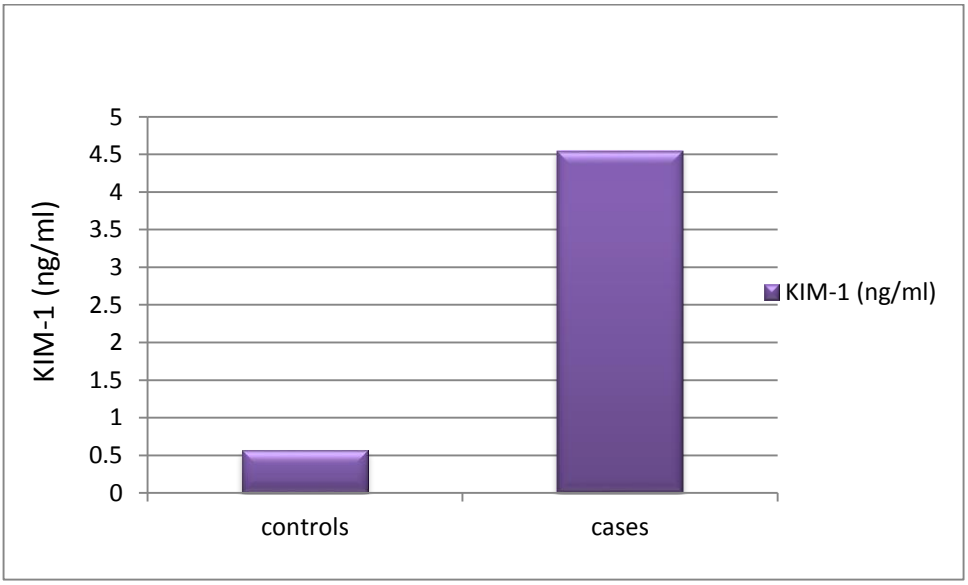


TABLE 9 - Comparison of urinary KIM-1 in different age groups among cases and controls with sepsis

Age group	Controls		Cases	
	Mean	S.D.	Mean	S.D.
<40 years	0.538	0.256	4.042	2.4217
≥40 years	0.571	0.202	5.008	2.1057
'p' value	0.754 Not significant		0.30 Not significant	

FIGURE 10

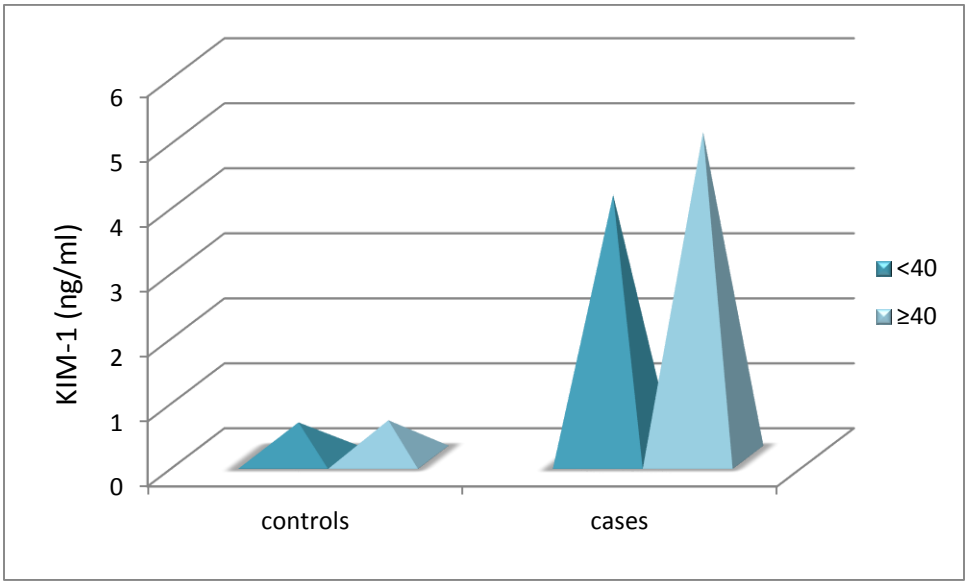


TABLE 10 - Comparison of urinary KIM-1 among males and females in cases and controls with sepsis

Sex	Controls		Cases	
	Mean	S.D.	Mean	S.D.
Males	0.557	0.199	4.306	2.33
Females	0.564	0.246	5.05	2.18
'p' value	**0.944** Not significant		**0.449** Not significant	

FIGURE 11

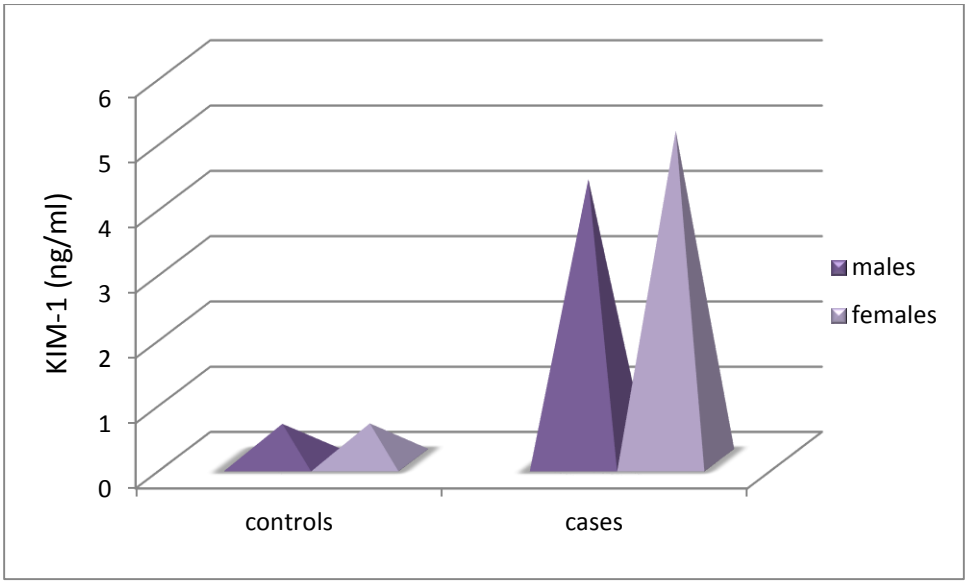


TABLE 11 - Comparison of serum creatinine in cases and controls with sepsis

Creatinine values on	Controls		Cases		'p' value
	Mean	S.D.	Mean	S.D.	
Day 1	0.86	0.191	1.072	0.499	0.056 Not significant
Day 3	1.034	0.195	3.656	2.585	<0.001 Significant

FIGURE 12

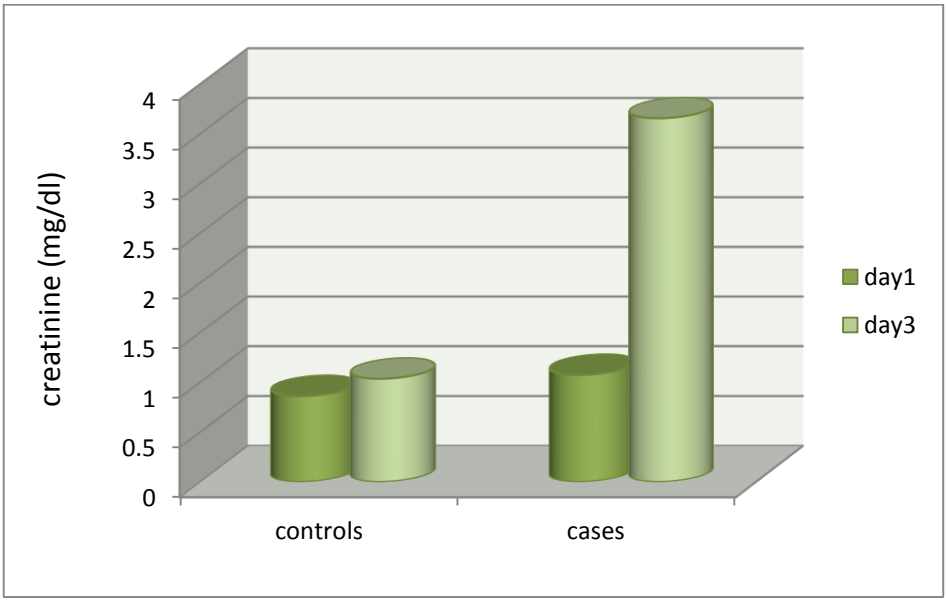


TABLE 12 - Comparison of serum urea in cases and controls with sepsis

Urea values on	Controls		Cases		'p' value
	Mean	S.D.	Mean	S.D.	
Day 1	32	7.836	36.44	8.347	0.0667 Not significant
Day 3	38.52	6.982	102.76	55.074	<0.001 Significant

FIGURE 13

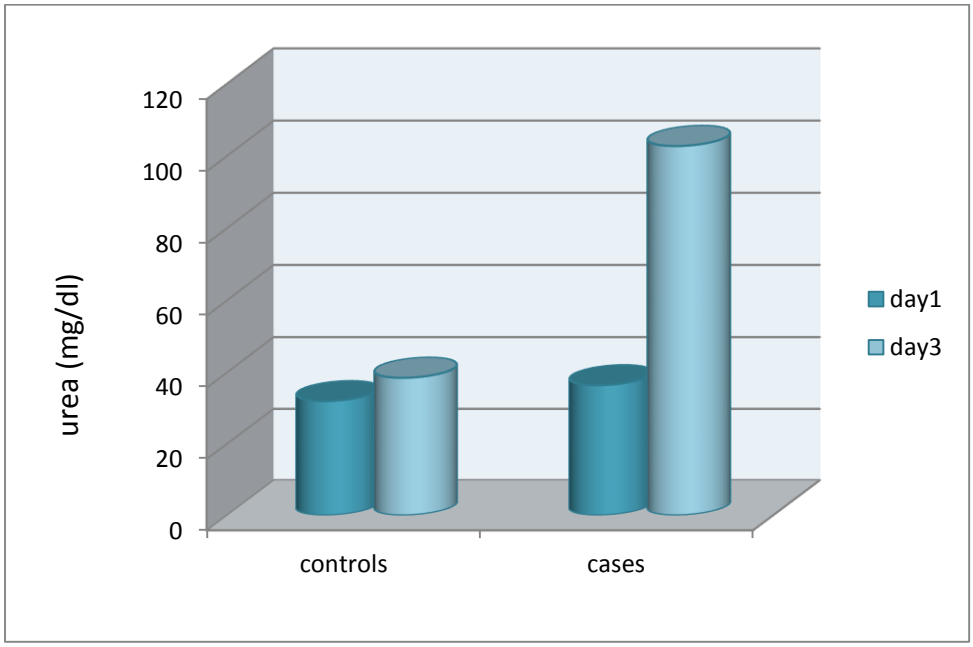


TABLE 13 - Comparison of urinary KIM-1 with serum creatinine and blood urea on day 1 in patients with sepsis

Day -1		Mean	S.D.	'p' value
KIM-1	Cases	4.544	2.268	<0.001 Significant
	Controls	0.56	0.216	
Serum creatinine	Cases	1.072	0.499	0.056 Not significant
	Controls	0.86	0.191	
Blood urea	Cases	36.44	8.347	0.0667 Not significant
	Controls	32	7.836	

TABLE 14 - Pearson's correlation coefficient between urinary KIM-1, serum creatinine and blood urea in patients with sepsis

Correlation between	Correlation coefficient	Correlation
KIM-1 and creatinine on day 1	0.439	Not correlated
KIM-1 and creatinine on day 3	0.888	**Correlated**
KIM-1 and urea on day 1	0.688	Correlated
KIM-1 and urea on day 3	0.871	**Correlated**

Table 15 : Distribution of snake bite cases who developed AKI according to KDIGO staging of AKI and urinary KIM-1 levels.

Stages of AKI	N	Mean KIM-1 level	Standard deviation	Standard error
Stage 1	8	2.025	0.656	0.232
Stage 2	8	4.2875	0.710	0.251
Stage 3	9	7.755	0.792	0.264

Table 16 : ANOVA test to test the difference in mean KIM-1 levels among groups with different stages of AKI following snake bite.

P value	**<0.001**
F statistic	**135.356**
Degree of freedom	**2**

Figure 14 :

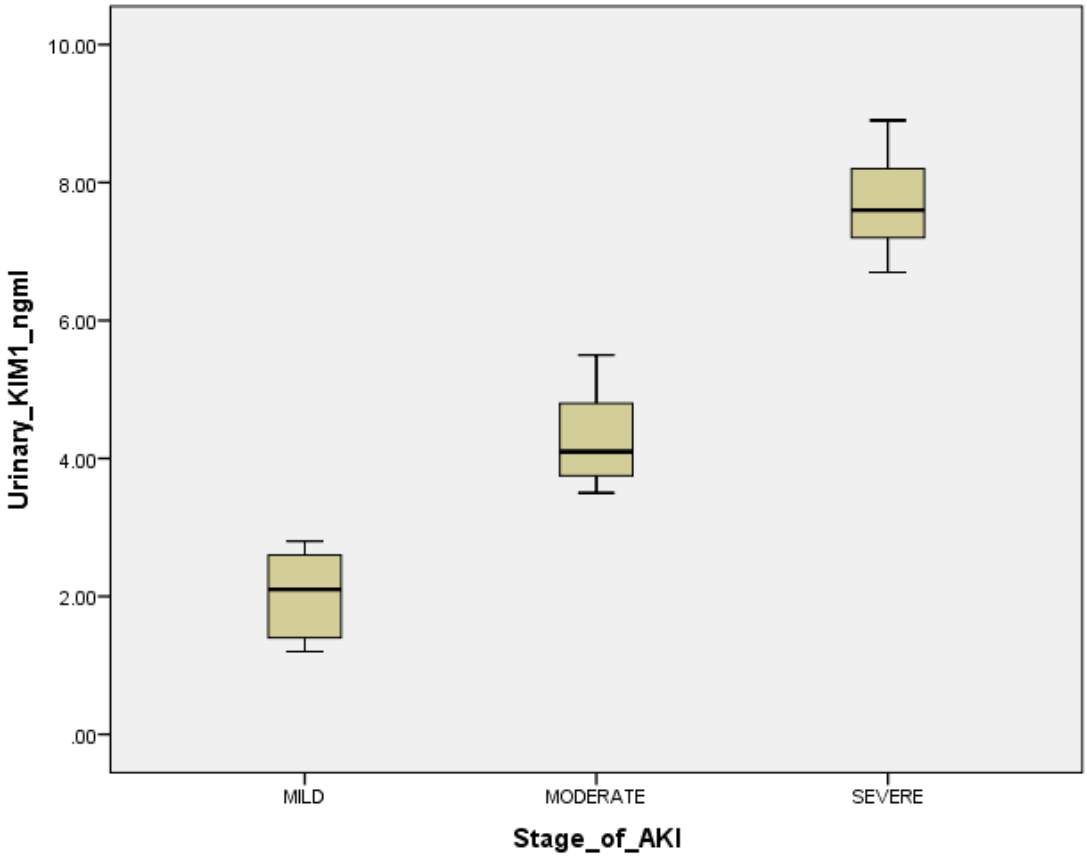


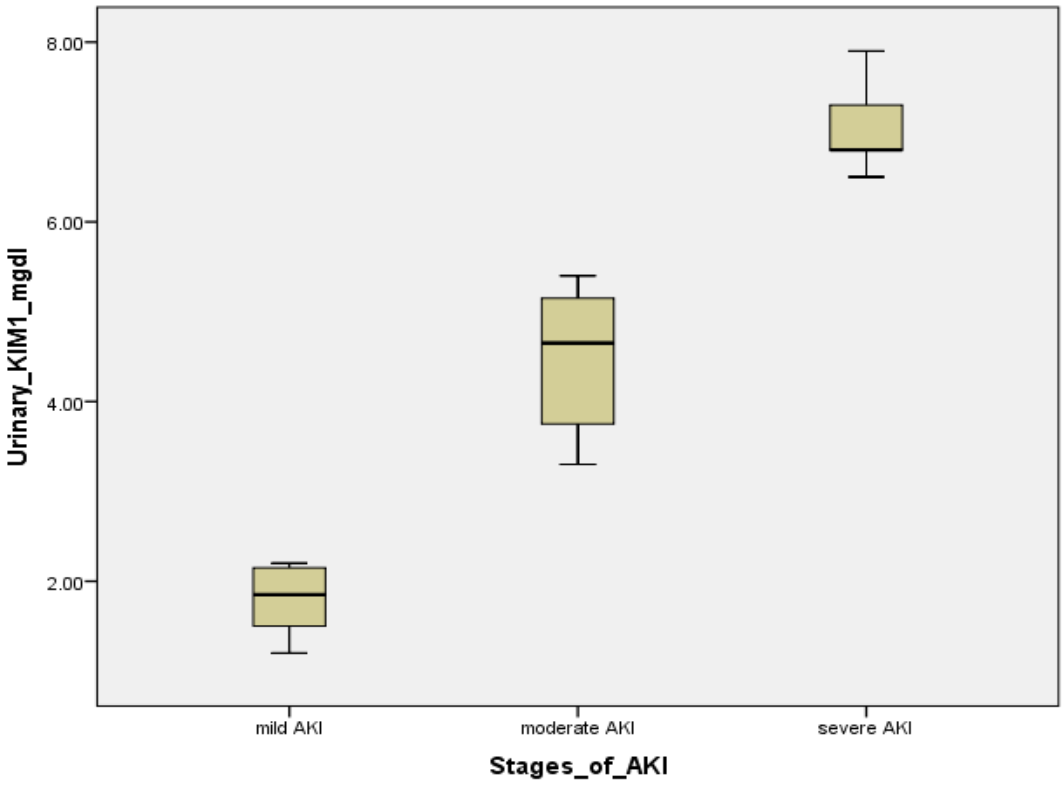
Table 17 :Distribution of sepsis cases who developed AKI according to KDIGO staging of AKI and urinary KIM-1 levels.

Stages of AKI	N	Mean KIM-1 level	Standard deviation	Standard error
Stage 1	8	1.800	0.374	0.132
Stage 2	8	4.475	0.785	0.277
Stage 3	9	7.0444	0.461	0.154

Table 18: ANOVA test to test the difference in mean KIM-1 levels among groups with different stages of AKI following sepsis.

P value	**<0.001**
F statistic	**183.214**
Degree of freedom	**2**

Figure 15



DISCUSSION

DISCUSSION

Acute Kidney Injury is a common cause of morbidity and mortality among hospitalized patients. Hence, sensitive biological markers for renal tubular injury are needed in order to detect early kidney injury and facilitate timely introduction of treatment. Till date, the standard test which is done to detect AKI is serum creatinine.

However, serum creatinine has shown numerous limitations as a marker to detect AKI, which affects its early diagnosis as well as prognosis.

Serum creatinine will be detected in serum only after 50% of renal cell death has occurred. It does not accurately depict kidney function until a steady state has been reached.

Serum creatinine levels are relatively insensitive to small changes in GFR and may lag behind changes in GFR by several days. Damage to renal tubules alone is not sufficient to result in a change in serum creatinine. Hence it does not allow for early detection of acute renal injury.

The change in serum creatinine does not discriminate the time and type of renal insult or the site and extent of glomerular or tubular injury.

To deal with these issues, better biological markers of AKI are needed, which should meet several requirements such as,

- Allow for early detection of kidney injury
- Identify the severity of kidney injury
- Provide a criteria for risk stratification and identify those patients who are at risk for AKI
- Guide timing of treatment
- Reflect improvement and deterioration of kidney injury.

This study evaluates the use of urinary Kidney Injury Molecule-1 (KIM-1) as a biomarker of AKI and for early diagnosis of AKI when compared to serum creatinine and blood urea.

In the present study, among patients who were admitted for snake bite, the mean value of urinary KIM-1 in those who developed AKI (cases) was significantly higher than that among those who did not develop AKI (control group).

Likewise, among patients who had sepsis, the mean value of urinary KIM-1 in cases was found to be significantly higher than that of control group.

Urinary KIM-1 was compared among age groups of <40 years and ≥40 years. Among patients with snake bite as well as sepsis, it was found that there was no significant difference in urinary KIM-1 values in different age groups among cases and controls. This shows that age doesn't have any influence on urinary KIM-1 values.

Further, when urinary KIM-1 was estimated and compared between males and females, among snake bite cases, it was found that urinary KIM-1 values are

significantly high among males when compared to females, whereas there was no significant difference between males and females among controls.

Among patients with sepsis, there was no significant difference in urinary KIM-1 values between males and females among cases, as well as between control males and control females. This implies that gender does not influence the values of urinary KIM-1 in patients with sepsis.

In this study, when serum creatinine was compared between cases and controls on day1. Both among patients with snake bite and sepsis it was found that there was no significant difference. On day 3, among patients with snake envenomation, serum creatinine is significantly elevated in cases than in controls, as also among patients with sepsis.

Similarly blood urea was compared among cases and controls on day 1. In patients with snake bite as well as sepsis, blood urea was not significantly elevated on day 1 between cases and controls. Whereas, on day 3, blood urea is elevated in both snake bite as well as sepsis cases than among controls.

This study shows that rise in serum creatinine and blood urea level is observed only on the 3rd day of nephrotoxic insult who developed acute kidney injury.

On day 1, when urinary KIM-1 was compared with serum creatinine and blood urea, it was found that among patients with snake bite, there was a significant rise in the levels of urinary KIM-1 among cases than controls with a ‘p’ value of < 0.001. But, there was no significant rise in levels of serum creatinine (‘p’ value 0.356) and blood urea (‘p’ value 0.607) between cases and controls on day 1.

Also among patients with sepsis, there was a significant rise in the levels of urinary KIM-1 among cases than controls with a 'p' value of < 0.001 on day 1. But there was no significant rise in levels of serum creatinine ('p' value 0.56) and blood urea ('p' value 0.667) between cases and controls on day 1.

This shows that urinary KIM-1 levels rise much earlier than the traditional biomarkers such as serum creatinine and blood urea.

In the present study, the correlation of urinary KIM-1 with traditional markers, serum creatinine and blood urea on day 1 and day 3 was determined using Pearson's correlation. It was found that in patients who developed AKI following snake envenomation, there was no significant correlation noticed between rise in urinary KIM-1 and serum creatinine as well as blood urea on the day of admission. Progressive kidney damage leading to rise in serum creatinine shows a significant positive correlation between urinary KIM-1 and serum creatinine, blood urea on day 3.

Among patients with sepsis who developed AKI, it was found that there was no significant correlation of urinary KIM-1 with serum creatinine on day1. But on day 3, there was a significant positive correlation. On day 1, although it was found that there was a significant positive correlation between urinary KIM-1 and blood urea, on day 3 urinary KIM-1 was more significantly correlated with blood urea.

The cases of snake bite and sepsis who developed AKI were categorized into three groups, stage 1, 2 and 3, on the basis of KDIGO staging for AKI. The descriptive statistics of urinary KIM-1 for each stage of AKI was determined. Further, ANOVA test was applied to test for the difference between the means of the three groups.

It was found that among snake bite cases, there was a significant difference between the mean of stage 1 AKI (2.025ng/ml). stage 2 AKI (4.287ng/ml) and stage 3 AKI (7.755ng/ml), with a 'p' value of <0.001. Likewise, among sepsis cases, there was a significant difference between the mean of stage 1 AKI (1.8ng/ml). stage 2 AKI (4.475ng/ml) and stage 3 AKI (7.044ng/ml), with a 'p' value of <0.001.

Thus in both snake bite and sepsis cases it was found that the levels of urinary KIM-1 increases with severity of AKI.

All these findings show that urinary KIM-1 is sensitive to minor disturbances in the renal function, specific and a noninvasive method for the early diagnosis and evaluation of acute kidney injury when compared with traditional markers such as serum creatinine and blood urea. [61] Urinary KIM-1 levels correlate with the degree of severity of tubular injury. In the setting of ischemic acute tubular necrosis, KIM-1 is an efficient marker to diagnose within 24 hours of kidney injury.[62,63]

In response to an acute insult to the renal tubules, the proximal tubular epithelial cells undergo dedifferentiation. Kidney Injury Molecule-1 expression is markedly upregulated in these dedifferentiated cells. Epithelial cellular stress leads to activation of Mitogen Activated Protein Kinase (MAPK) pathway, following which the heavily glycosylated KIM-1 ectodomain is shed from the cellular surface into the tubular lumen by the action of Matrix Metalloproteinases (MMPs). This leads to release of a 90kDa soluble form which is excreted in urine.

The characteristic features of KIM-1 which make it an ideal biomarker include:

- It is specifically expressed only in the injured proximal tubular cells of kidney and is virtually absent in normal healthy kidneys. It persists till the injured cells are completely recovered.
- The rapidly cleaved ectodomain which sheds into the tubular lumen makes it detectable in urine. Urinary KIM-1 correlates with tissue KIM-1 as well as the severity of kidney damage.
- This qualifies urinary KIM-1 as a non-invasive, sensitive, rapid and reproducible method to evaluate renal injury.[64,65]
- Urinary KIM-1 serves as a prognostic indicator of rate of decline of renal function, irrespective of renal pathology.
- The detection of KIM-1 in urine within 12 hours following ischemia or toxic injury makes it an early diagnostic indicator compared to the traditional biomarkers such as blood urea nitrogen and serum creatinine.[66]
- The appearance of KIM-1 before the detection of lethal injury to proximal tubular epithelial cells allows for timely reversal and treatment of kidney injury.[53]
- Since KIM-1 is found to be stable in urine even after repeat freeze-thaw cycles, a methodological advantage is that no stabilizing buffer or protease inhibitor is required to prevent its degradation while collecting urine samples.[20,67]

- KIM-1 detected by ELISA is associated with minimal interference by other urinary components of the diseased patient and is unaffected by physic-chemical changes in urine.[56]
- KIM-1 is also a tool to evaluate renal injury in biopsy specimen using immunohistochemical methods, and its upregulation has been correlated with inflammation and tubule-interstitial fibrosis.
- The behavior of KIM-1 in human mirrors as in animals. Thus it is a 'true translational biomarker' which can be used in drug development, evaluation of toxicity of new candidate therapeutics and safety monitoring of kidneys.[3,31] The Food and Drug Administration of United States (FDA) and European Medicines Agency (EMEA) has qualified KIM-1 for the preclinical assessment of nephrotoxicity to improve the monitoring of kidney safety.[68]

The utility of KIM-1 has also been found in several other conditions such as

1) Chronic kidney disease
2) Diabetic glomerulopathy .[9]
3) Acute or chronic renal transplant dysfunction.[61]
4) Biomarker for renal cell carcinoma[20]
5) Kidney injury in chidren undergoing cardiac surgery[3]
6) IgA nephropathy associated with tubule-interstitial injury[69]

CONCLUSION

CONCLUSION

This study on patients with snake envenomation and sepsis shows that urinary Kidney Injury Molecule-1 is a promising early predictive bio-marker of acute kidney injury when compared to the traditional biomarkers such as serum creatinine and blood urea.

This novel bio- marker might facilitate earlier diagnosis of acute kidney injury and management decision including administration of specific preventive and therapeutic strategies, potentially resulting in fewer morbidity and mortality.

LIMITATIONS

This study had the following limitations :

1. The sample size was small.
2. Urinary KIM-1 was analyzed only on the day 1 of admission. It is not clear if KIM-1 level in the urine shows fluctuations with time.

ANNEXURE

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BIBLIOGRAPHY

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STUDY OF URINARY LEVEL OF KIDNEY INJURY MOLECULE-1 (KIM-1) IN ACUTE KIDNEY INJURY –PROFORMA

NAME OF THE PATIENT :

AGE/SEX :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

Ht: Wt: BMI: BP: PR:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

INVESTIGATIONS :

1.BLOOD UREA:

2.SERUM CREATININE

3. URINARY KIDNEY INJURY MOLECULE-1:

CONSENT FORM

Dr .NEETHU VARGHESE post graduate student in the Department of Biochemistry, Thanjavur medical college, Thanjavur is doing a dissertation on Study of Urinary level of Kidney Injury Molecule-1 (KIM-1) in Acute Kidney Injury. The procedure has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature :

Name:

Place:

MASTER CHART I - SNAKE BITE CONTROLS

S.No	AGE (years)	SEX	Type of Snake	Blood Pressure (mmHg)	Pulse Rate (per minute)	Clotting time (minutes)	Urinary KIM-1 (ng/ml)	Serum creatinine (mg/dl)		Blood urea (mg/dl)		Stage of AKI
								Day 1	Day 3	Day 1	Day 3	
1	75	M	Viper	110/70	76	18	0.4	1.1	1	32	38	No AKI
2	43	M	Viper	112/68	82	>20	0.6	0.8	1.1	36	45	No AKI
3	34	F	Unknown	120/80	73	6	0.6	0.6	0.7	29	24	No AKI
4	20	M	Unknown	120/80	72	10	0.2	0.7	1	35	40	No AKI
5	45	F	Unknown	110/70	70	16	0.7	0.9	1.3	28	64	No AKI
6	40	M	Unknown	120/70	79	7	0.9	0.6	0.8	29	32	No AKI
7	65	F	Unknown	130/80	80	9	0.5	1	1.4	30	45	No AKI
8	18	F	Viper	110/68	83	15	0.2	0.6	0.8	38	68	No AKI
9	19	M	Viper	110/80	71	8	0.9	0.6	0.8	32	40	No AKI
10	18	M	Unknown	120/80	74	6	0.4	0.6	0.7	20	38	No AKI
11	21	M	Unknown	110/70	74	7	0.8	0.6	0.7	21	40	No AKI
12	55	M	Unknown	120/80	76	10	0.4	1.2	1.3	44	47	No AKI
13	73	M	Unknown	120/70	78	12	0.7	1	1.2	34	36	No AKI
14	23	F	Unknown	120/80	70	9	0.1	0.8	1	24	32	No AKI
15	62	F	Unknown	110/68	76	18	0.7	0.9	1.1	36	42	No AKI
16	35	F	Unknown	108/70	82	10	0.6	0.7	0.8	28	22	No AKI
17	22	M	Viper	120/70	80	9	0.9	1.1	1.5	40	38	No AKI
18	43	F	Viper	110/80	76	12	0.6	0.7	1.1	29	43	No AKI
19	41	M	Unknown	110/70	78	8	0.3	0.9	1.1	30	35	No AKI
20	22	F	Unknown	110/80	75	15	0.5	0.8	1.1	36	46	No AKI
21	18	F	Unknown	110/70	76	19	0.8	0.6	0.8	33	46	No AKI
22	42	F	Unknown	110/70	76	5	0.7	0.9	1.25	48	43	No AKI
23	32	M	Unknown	112/78	84	18	0.2	0.7	0.9	30	35	No AKI
24	54	M	Viper	130/70	77	15	0.3	1.1	1.3	42	38	No AKI
25	37	F	Unknown	120/70	84	14	0.6	0.7	1	29	38	No AKI

MASTER CHART I - SNAKE BITE CASES

S.No	AGE (years)	SEX	Type of Snake	Blood Pressure (mmHg)	Pulse Rate (per minute)	Clotting time (minutes)	Urinary KIM-1 (ng/ml)	Serum creatinine (mg/dl)		Blood urea (mg/dl)		Stage of AKI
								Day 1	Day 3	Day 1	Day 3	
1	22	F	Unknown	120/80	80	7	1.2	0.6	0.9	26	42	1
2	60	F	Unknown	108/72	90	17	3.5	0.9	1.9	34	80	2
3	24	M	Unknown	102/72	82	>20	7.2	0.7	3.2	40	154	3
4	48	M	Viper	110/70	74	>20	7.5	0.8	6.4	46	160	3
5	45	F	Viper	110/80	75	10	4.5	0.8	2.1	28	66	2
6	40	M	Unknown	110/70	75	>20	8.2	0.8	7.4	29	112	3
7	20	F	Viper	120/70	76	13	1.8	0.9	1.35	38	82	1
8	39	M	Unknown	110/70	72	11	4.3	1.2	2.4	27	54	2
9	40	F	Viper	120/70	70	9	3.7	1.2	2.4	46	60	2
10	20	F	Unknown	110/64	90	>20	3.9	0.9	2	38	78	2
11	37	M	Unknown	106/66	94	>20	6.8	0.8	6.6	40	103	3
12	33	M	Viper	120/80	80	6	1.5	0.8	1.2	32	44	1
13	45	M	Viper	110/70	72	16	5.5	0.9	2.5	24	74	2
14	24	M	Unknown	110/80	78	15	6.7	0.8	3.1	42	150	3
15	49	M	Unknown	110/70	76	>20	8.2	0.9	6.2	44	154	3
16	44	F	Unknown	120/80	74	15	2.4	0.8	1.4	27	66	1
17	63	F	Unknown	120/80	72	7	1.3	1.1	1.65	34	44	1
18	38	M	Unknown	120/70	73	>20	8.7	0.9	7.5	30	130	3
19	38	M	Unknown	120/84	82	7	5.1	1.2	2.4	30	70	2
20	20	M	Unknown	120/80	80	6	2.8	0.7	1.05	34	48	1
21	16	M	Unknown	110/80	78	10	2.4	0.6	0.9	20	39	1
22	22	F	Viper	112/70	90	15	3.8	0.8	2.1	37	76	2
23	35	M	Viper	120/80	78	12	7.6	0.7	6.6	36	104	3
24	22	M	Unknown	110/80	72	6	2.8	0.7	1.05	22	39	1
25	45	M	Viper	140/90	96	19	8.9	1.1	4.5	37	138	3

MASTER CHART II - SEPSIS CONTROLS

S. No	Age (years)	Sex	Septic foci	Blood pressure (mmHg)	Pulse rate (/min)	Temperature (°F)	Urinary KIM-1 (ng/ml)	Serum creatinine (mg/dl)		Blood urea (mg/dl)		Stage of AKI
								Day 1	Day 3	Day 1	Day 3	
1	88	M	Pyelonephritis	130/80	85	100.5	0.7	0.8	1	38	45	No AKI
2	87	M	Pneumonitis	130/80	83	101.2	0.9	0.6	0.7	34	42	No AKI
3	24	M	Encephalitis	110/70	76	100.6	0.3	0.6	0.8	26	32	No AKI
4	55	M	Pyelonephritis	110/60	96	101	0.5	1	1.2	40	45	No AKI
5	27	F	Puerperal sepsis	100/68	94	95.9	0.6	1	1.3	44	48	No AKI
6	21	F	Meningitis	110/80	76	101	0.7	0.6	0.9	22	30	No AKI
7	68	M	Pneumonitis	110/60	84	96.3	0.5	0.9	1.1	19	45	No AKI
8	45	F	Appendicitis	120/70	76	96.2	0.6	0.8	0.9	26	28	No AKI
9	42	M	Pneumonitis	140/90	112	101	0.7	0.9	1.3	32	40	No AKI
10	80	M	Pneumonitis	110/80	78	100.7	0.8	0.7	1	32	40	No AKI
11	85	M	Pneumonitis	140/90	112	100.5	0.7	0.6	0.85	36	40	No AKI
12	52	M	Puerperal sepsis	100/60	98	100.4	0.3	0.8	1	40	50	No AKI
13	35	M	Puerperal sepsis	100/60	96	100.5	0.3	1.1	1.2	46	40	No AKI
14	40	F	Pyelonephritis	100/58	106	100.9	0.9	0.9	1.1	38	40	No AKI
15	40	M	Pneumonitis	90/60	112	100.7	0.6	0.8	1.1	21	30	No AKI
16	44	F	Pyelonephritis	100/60	108	101.1	0.3	0.9	1.1	26	32	No AKI
17	23	F	Meningitis	120/80	76	101.2	0.2	0.7	0.9	24	28	No AKI
18	42	F	Appendicitis	110/70	74	100.6	0.4	0.9	0.8	26	28	No AKI
19	40	M	Encephalitis	120/70	72	101.2	0.6	1.1	1.2	46	50	No AKI
20	66	M	Pancreatitis	100/60	86	96.3	0.6	1.1	1.2	30	46	No AKI
21	52	M	Meningitis	100/62	90	96.1	0.3	1.1	1.2	30	35	No AKI
22	38	F	Pyelonephritis	96/60	110	101.3	0.5	1.1	1.4	36	40	No AKI
23	23	F	Puerperal sepsis	100/70	106	101.4	0.9	1.2	1.1	36	40	No AKI
24	32	F	Puerperal sepsis	96/60	79	96.4	0.8	0.7	0.8	28	34	No AKI
25	43	F	Puerperal sepsis	100/70	92	96.4	0.3	0.6	0.7	24	35	No AKI

MASTER CHART II - SEPSIS CASES

S. No.	Age	Sex	Septic foci	Blood pressure (mmHg)	Pulse rate (/min)	Temperature (°F)	Urinary KIM-1 (mg/dl)	Serum creatinine (mg/dl)		Blood urea (mg/dl)		Stages of AKI
								Day 1	Day 3	Day 1	Day 3	
1	65	M	Pancreatitis	100/60	92	100.7	7.5	0.9	8.6	42	187	3
2	50	M	Meningitis	100/64	94	100.5	6.8	0.9	6.1	42	167	3
3	38	M	Pancreatitis	120/70	75	96.4	3.7	0.6	1.2	29	62	2
4	25	F	Puerperal sepsis	100/70	91	96.2	7.2	0.8	6.5	36	169	3
5	41	F	Pyelonephritis	96/60	102	96	6.5	0.9	3.3	39	153	3
6	28	F	Puerperal sepsis	100/60	96	101.3	1.9	0.8	1.3	40	42	1
7	32	F	Puerperal sepsis	108/70	87	101.1	2.2	0.9	1.4	26	44	1
8	38	M	Encephalitis	130/80	110	96.1	1.6	1.1	1.8	41	80	1
9	21	F	Puerperal sepsis	110/70	96	100.4	5.4	1.2	3.1	41	148	2
10	63	M	Pneumonitis	100/70	110	96.2	6.8	0.8	8.4	40	180	3
11	25	M	Encephalitis	110/70	82	100.4	1.8	0.9	1.6	30	48	1
12	35	M	Pancreatitis	110/70	84	96.5	3.3	0.7	2	30	60	2
13	63	M	Puerperal sepsis	100/60	76	96.3	6.6	0.9	6.4	34	170	3
14	53	M	Pyelonephritis	110/70	78	96.1	3.8	1.2	3.3	38	112	2
15	65	M	Pnemonitis	130/90	84	100.4	1.2	0.8	1.4	20	40	1
16	40	M	Pneumonitis	120/80	70	101.5	5.1	0.8	2.2	32	60	2
17	22	F	Puerperal sepsis	110/60	84	101.4	4.8	1.1	3.2	40	150	2
18	25	F	Puerperal sepsis	100/70	88	96.4	7.9	2.4	7.5	60	120	3
19	53	M	Pyelonephritis	100/60	92	101	2.2	1	1.3	28	35	1
20	25	M	Meningitis	100/62	98	101.3	1.4	0.7	0.9	24	40	1
21	45	M	Pyelonephritis	100/70	95	96.4	6.8	1.4	6.4	42	160	3
22	67	F	Puerperal sepsis	96/60	85	101	4.5	1.2	2.7	34	67	2
23	54	M	Pyelonephritis	100/70	88	101.3	5.2	1.1	2.5	38	73	2
24	37	M	Meningitis	110/60	78	101	7.3	2.8	7.2	49	154	3
25	54	M	Pyelonephritis	100/62	91	101.3	2.1	0.9	1.1	36	48	1